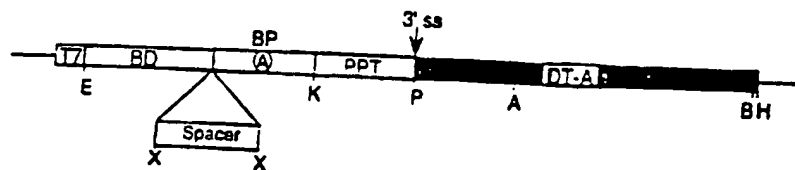


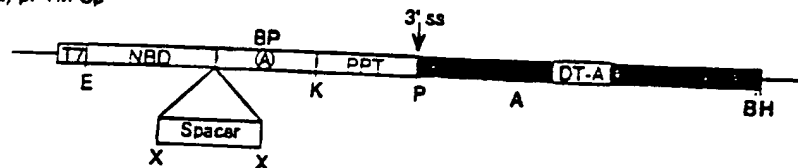
FIGURE 1A:



(B) (1) pPTM+Sp



(2) pPTM-Sp



(C)

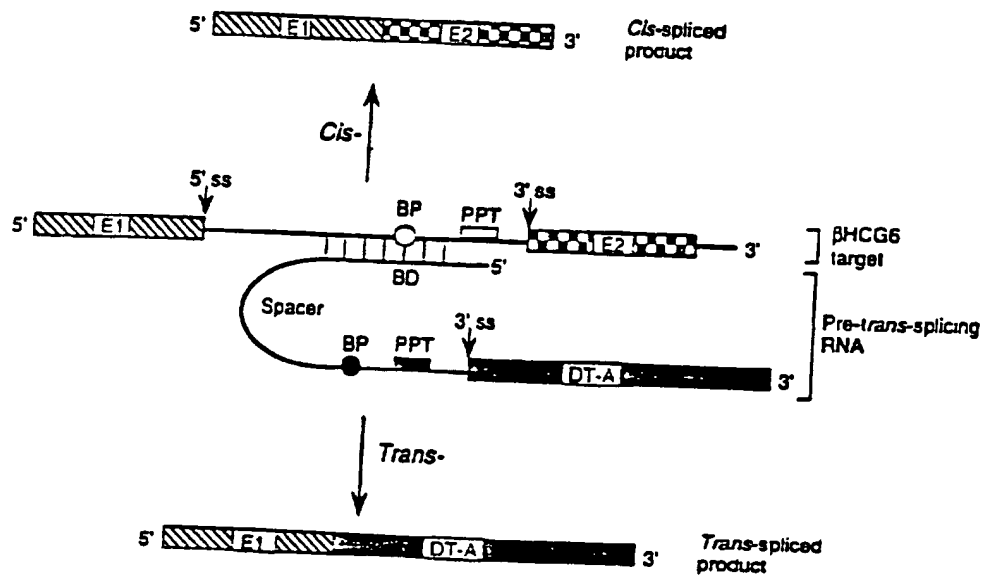
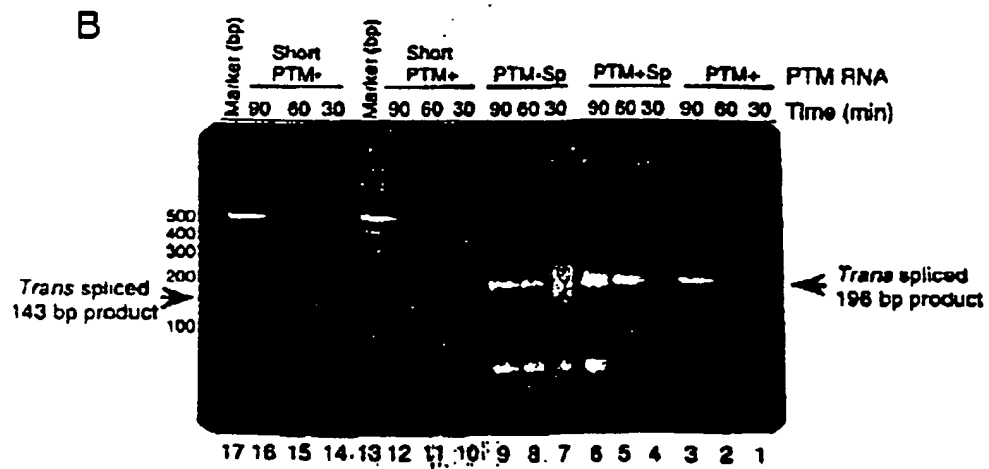
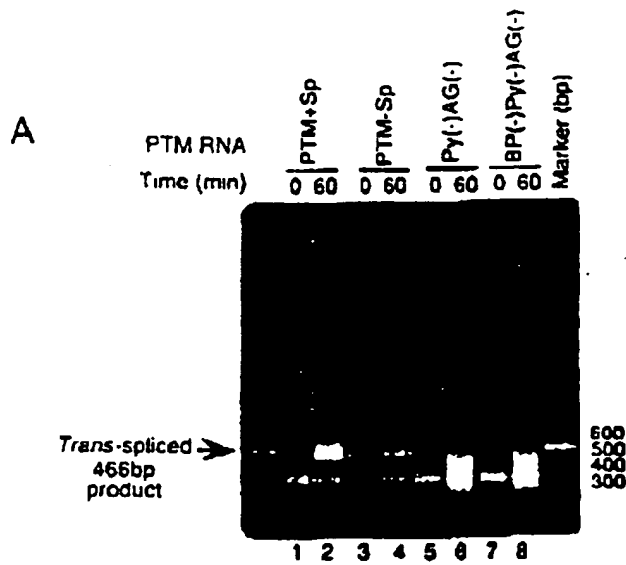


Figure 1 B-C



[illegible]

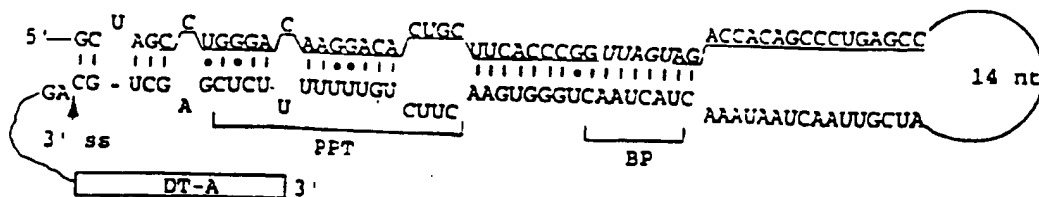
Exon 1 of BHC6

1st coding nucleotide of DT-A

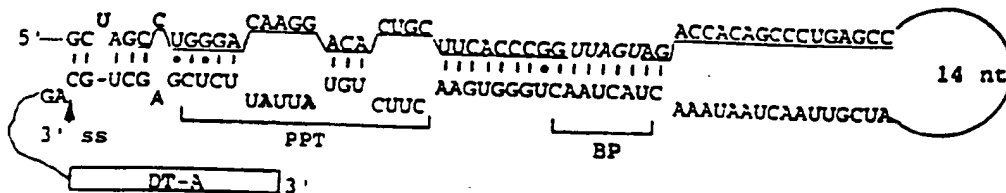
[illegible]

(A)

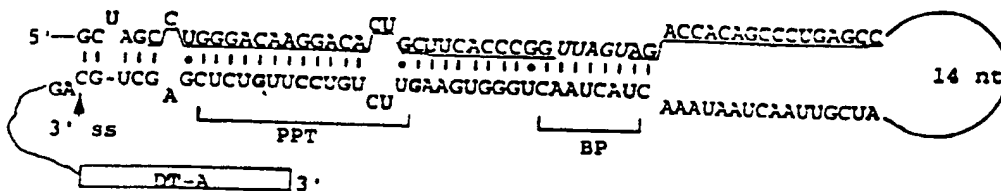
1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:



(B)

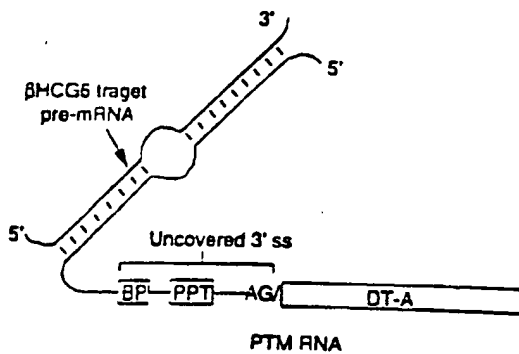


Figure 4A-B

(C)

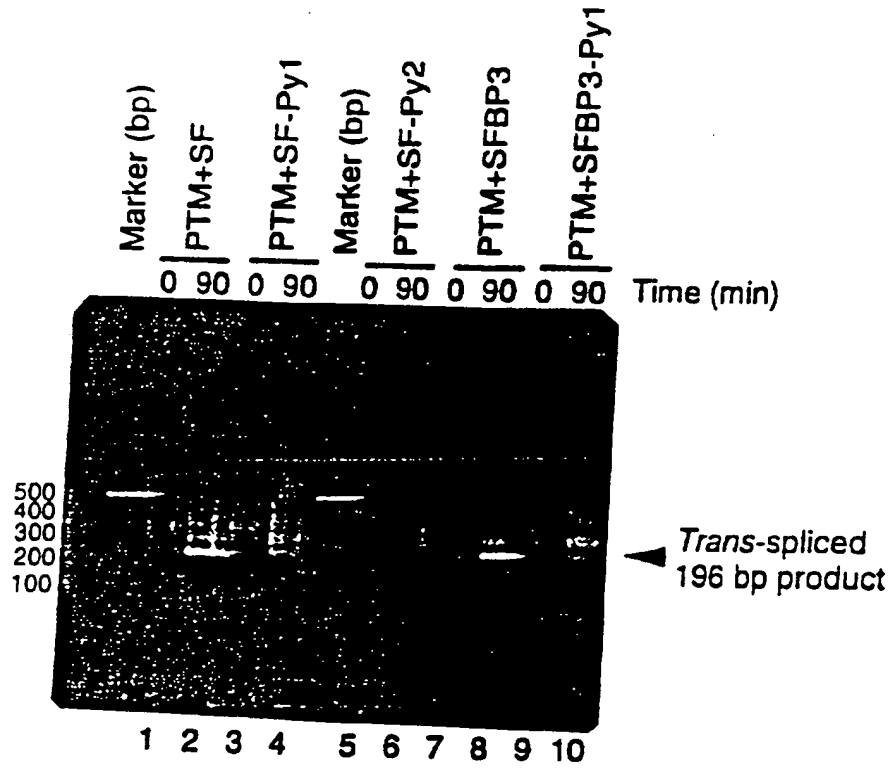


Figure 4c

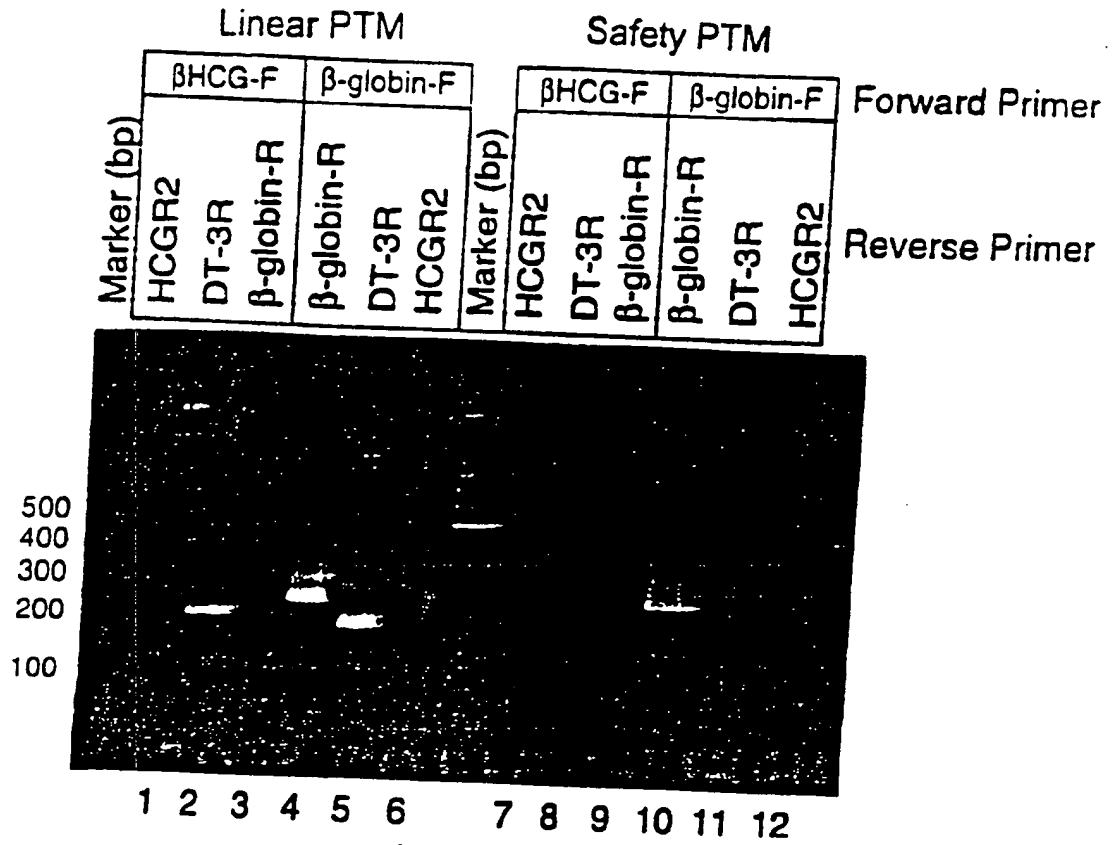


Figure 5

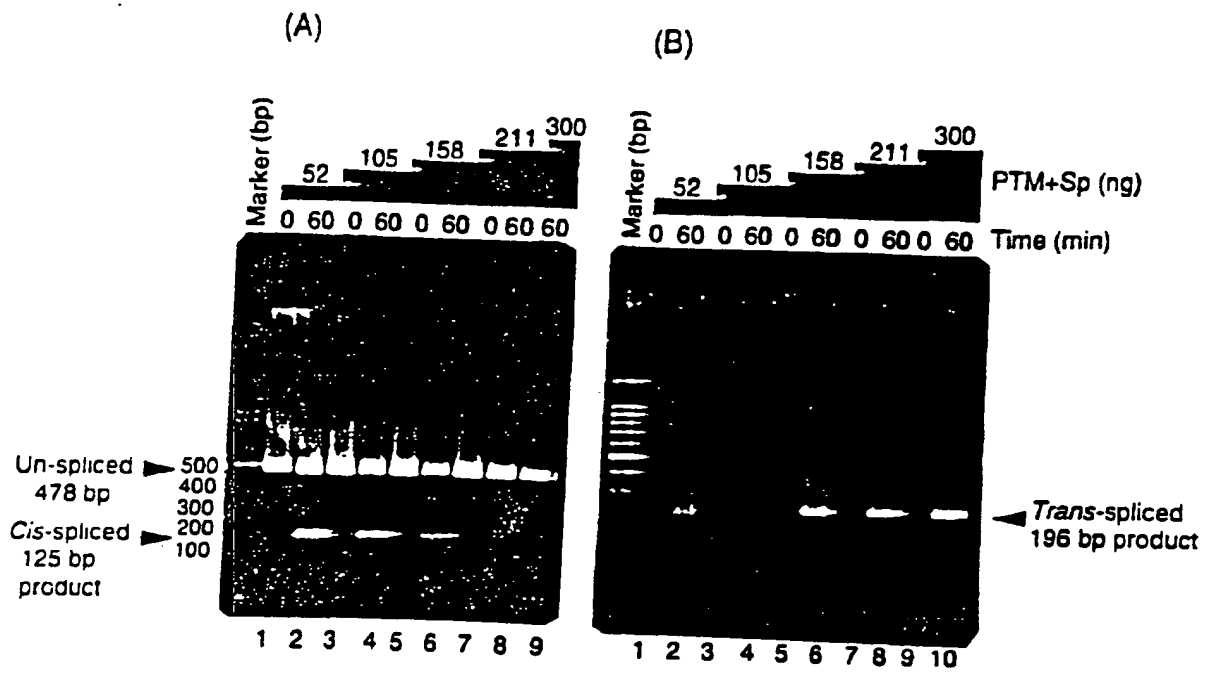
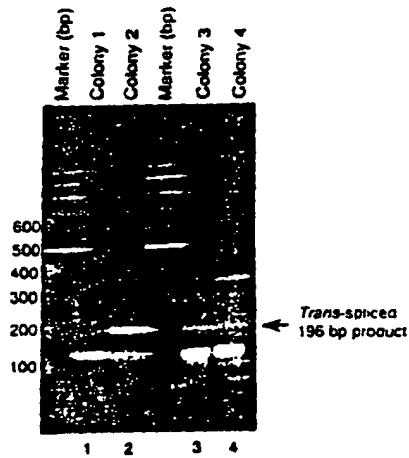


Figure 6

Figure 7

(A)



(B)

Exon 1 of β HCG6 ↓
 5'-CAGGGGACGCACCAAGGATGGAGATGTTCCAG-GGCGCTGATGATGTTGTT-
 ↑ 1st coding nucleotide of DT-A
 GATTCTTCTTAAATCTTTTGTGATGGAAAACCTTTCTTCGTACCACGGGACTA
 AACCTGGTTATGTAGATTCCATTCAAAAA-3'

Double Splicing Pre-therapeutic RNA

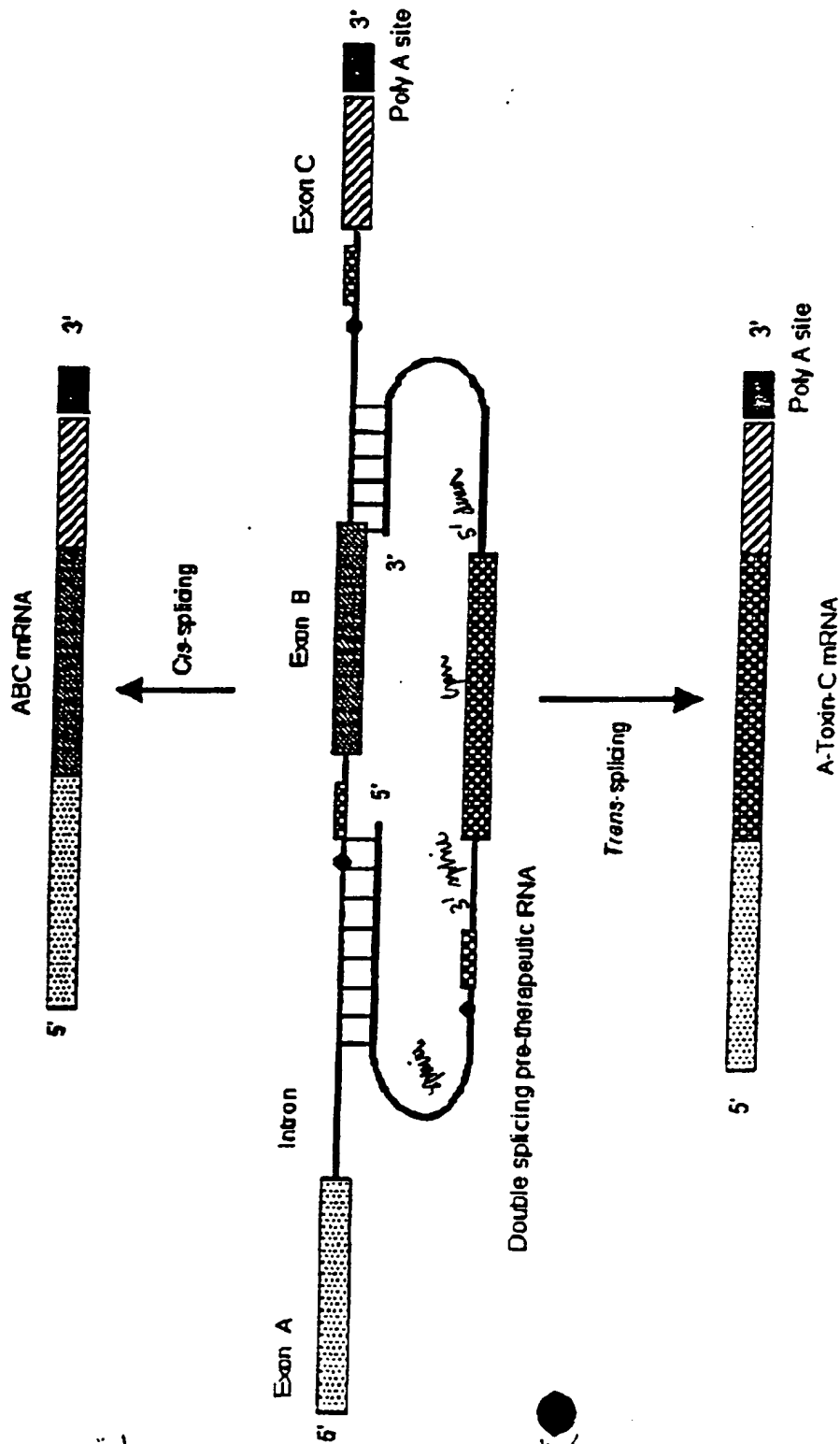


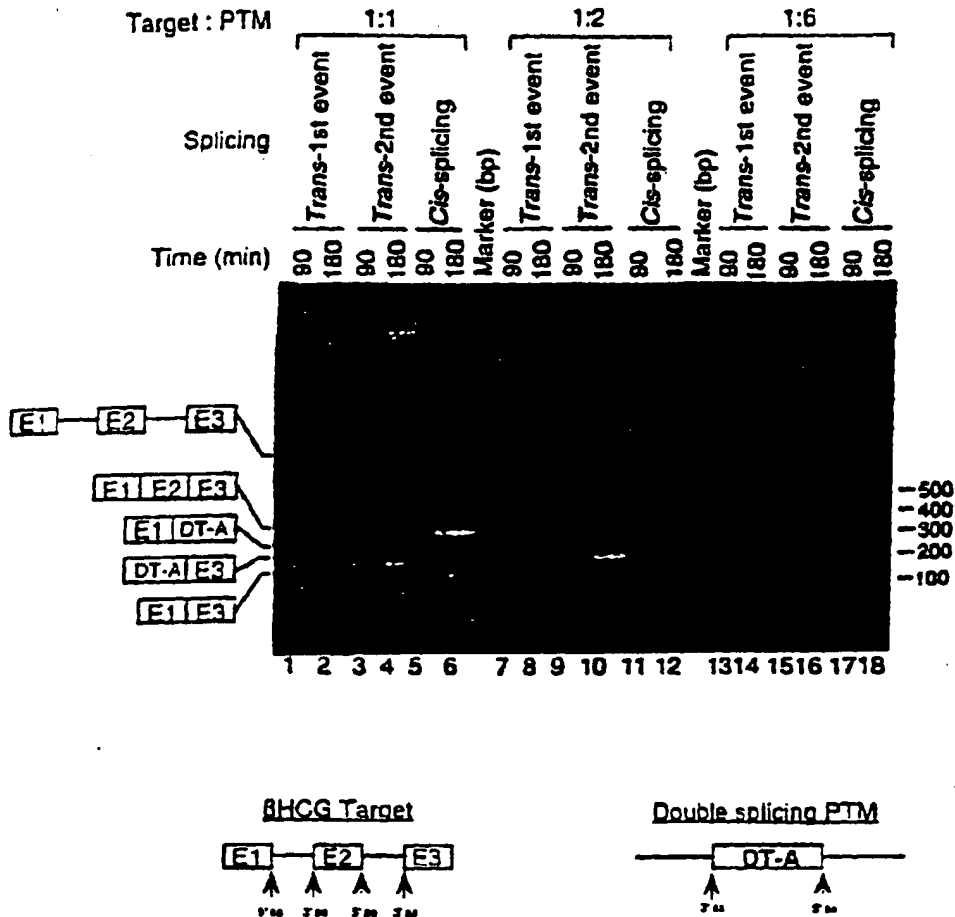
Figure 8A

31304B-A

(Sheet 12 of 58)

Selective *Trans*-splicing of a Double Splicing PTM

(3' ss of PTM to 5' ss target and, 5' ss of PTM to 3' ss of target)



Cis-spliced products

E1 E2 E3 = Normal *cis*-splicing (277bp)

E1 E3 = Exon skipping (110bp)

Trans-spliced products

E1 OT-A = 1st event, 196bp. *Trans*-splicing between 5' ss of target & 3' ss of PTM.

OT-A E3 = 2nd event, 161bp. *Trans*-splicing between 3' ss of target & 5' ss of PTM.

Figure 8B

31304B-A
(Sheet || Of 58)

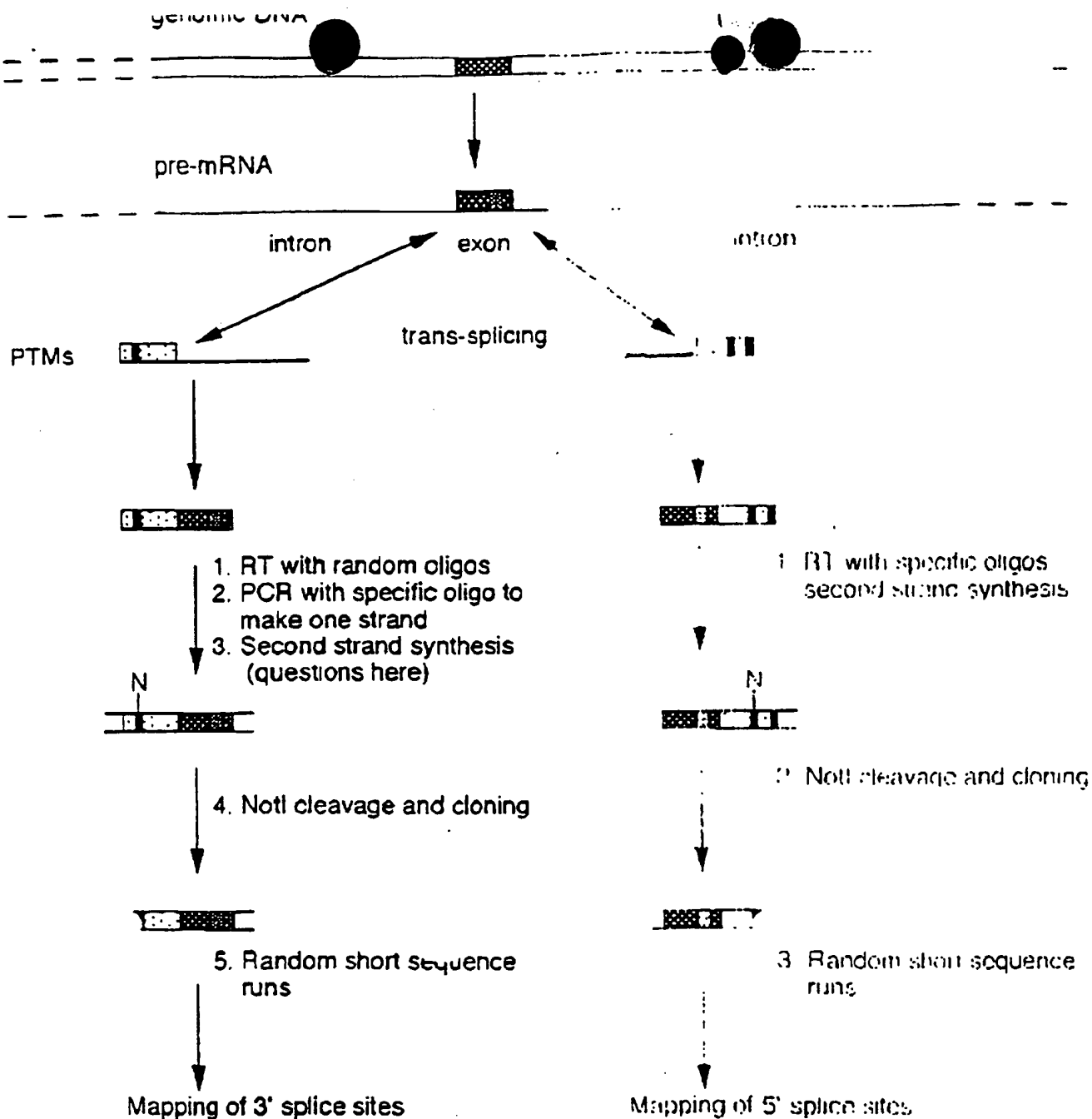


FIGURE 9

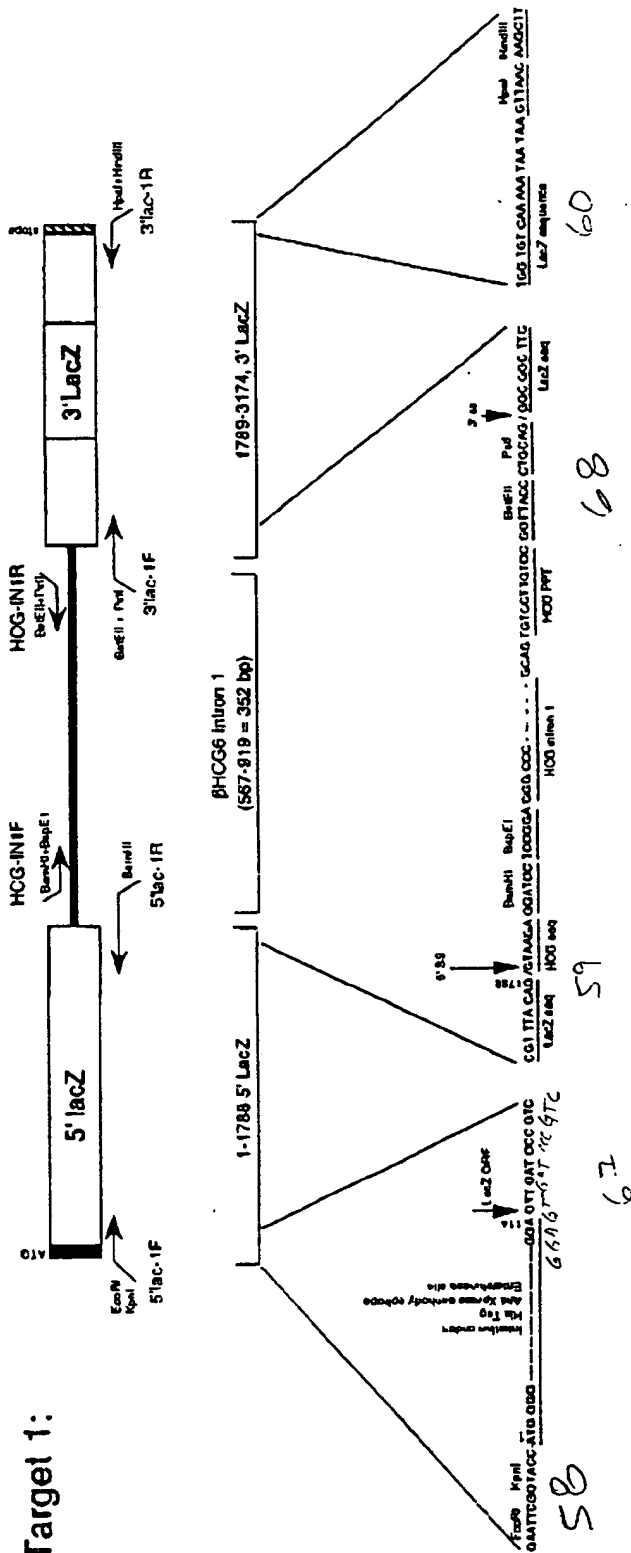
31304B-A
(Sheet 12 Of 58)

LacZ ~~Model~~ Constructs

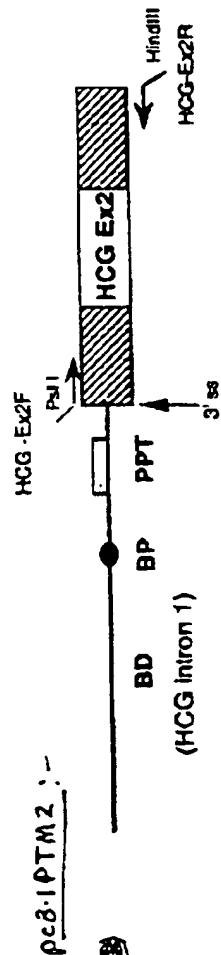
Knock Out

pc3.1 Lac-TI

Target 1:



PTMS



pc3.1PTM2 :-

BD BP PPT

(HCG Intron 1)

Restoration of β -Gal activity by SMaRT (Spliceosome Mediated RNA *Trans*-splicing)

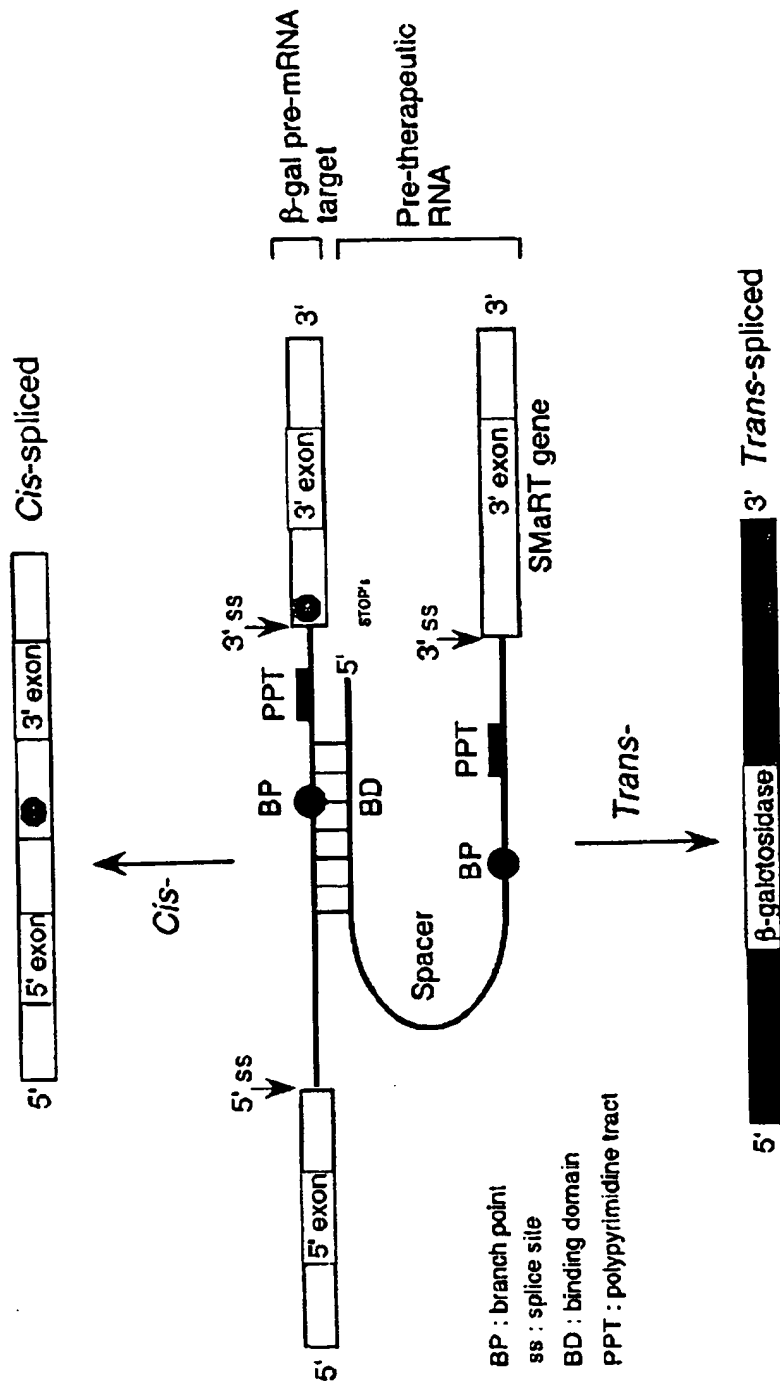


Figure 10B

31304 B-A
(Sheet 14 of 20)

31304 B-A
(Sheet 15 of 58)

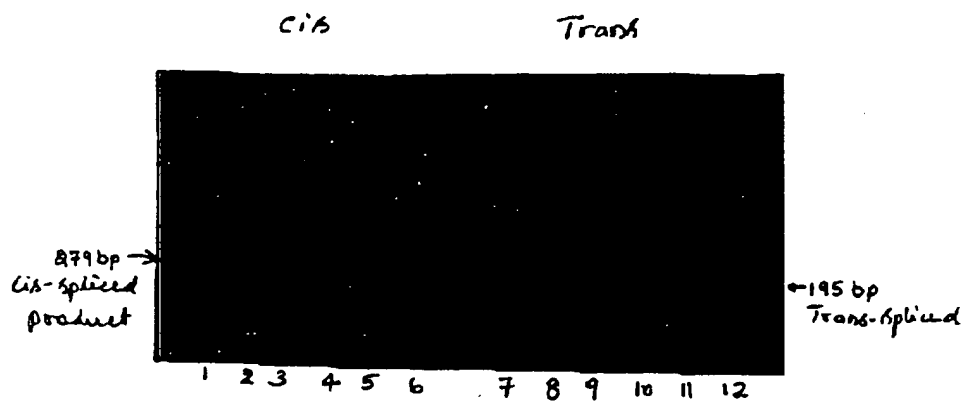


FIGURE 11A

Figure 11B

31307 15-11
(Sheet 17 of 58)

11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

FIGURE 11C

Nucleotide Sequence Demonstrating that
Trans-splicing is Accurate

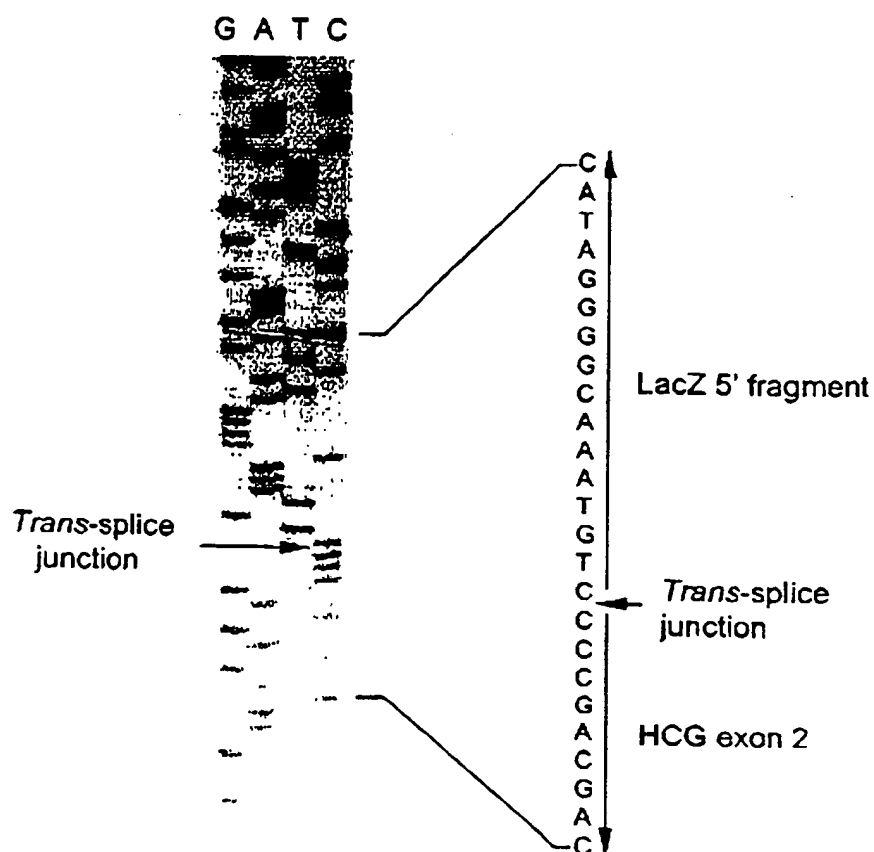


FIGURE 12 A

31304-B-A
(Sheet 18 of .58)

(1) Nucleotide sequences of the cis-spliced product (285 bp) :

BioLac-TR1

GGCTTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTG

Splice junction

CGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/GGCGGCTTCGTCTAATAATG

GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTTGGTCGGCTTACGGCGGTGATTT

Lac-TR2

TGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGGCGACCGCACGCCGCATCCAG

(2) Nucleotide sequences of the trans-spliced product (195 bp)

BioLac-TR1

GGCTTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGTCTTG

Splice junction

CGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/GGGCTGCTGCTGTTGCTGCTGCT

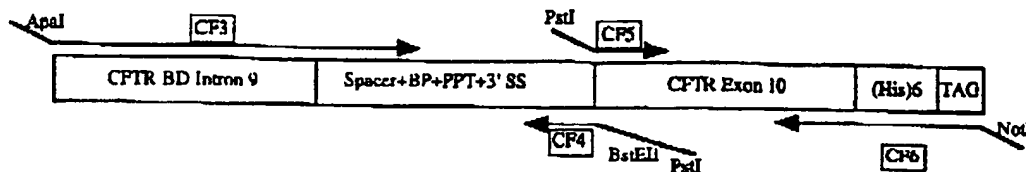
HCGR2

GAGCATGGGCGGGACATGGGCATCCAAGGAGCCACTTCGGCCACGGTGCCG

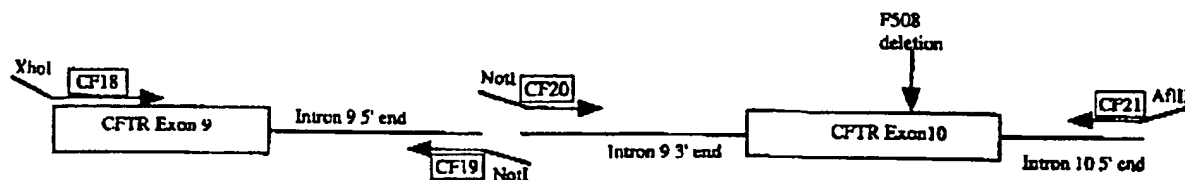
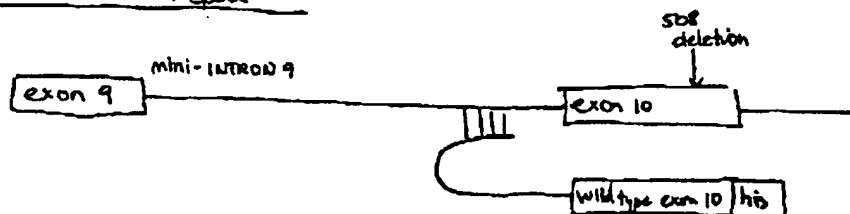
Figure 12 B

31304-B-A
(Shot 19 of 58)

CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target - Construction

TRANS-SPLICING RepairBinding
of
PTM to TARGET

↓ splicing

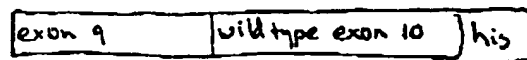
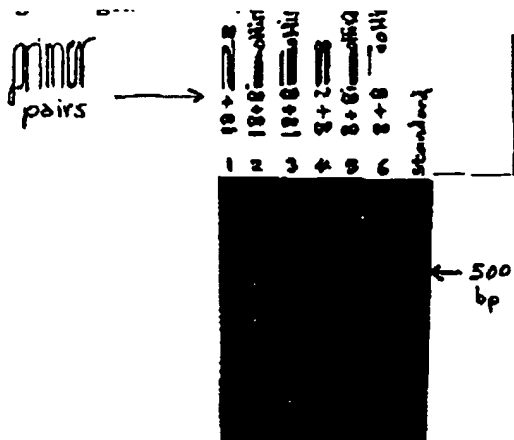


Figure 13

31304-B-A
(shut 2004.58)

Figure 14

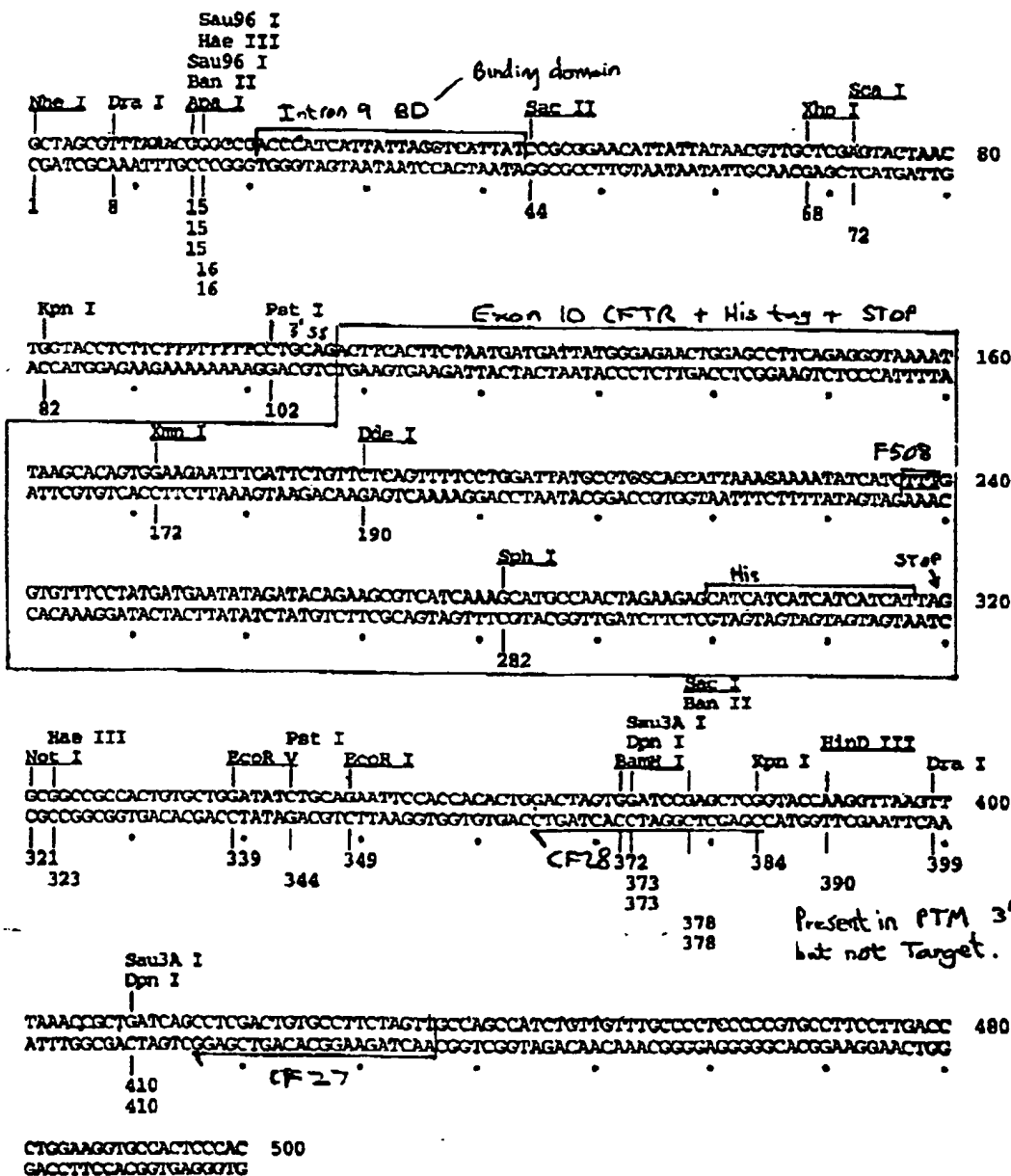


31304 B-A
(Sheet 21 of 58)

FIGURE 15

DNA sequence 500 b.p. GCTAGCGTTTAA ... TGCCACTCCAC linear

Positions of Restriction Endonucleases sites (unique sites underlined)

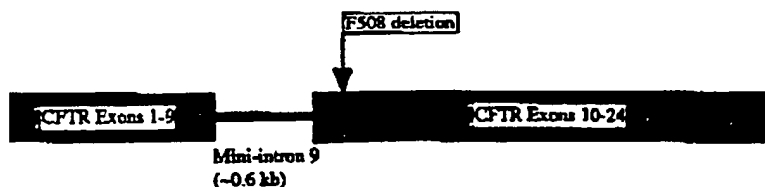


EXPERIMENT 2

Repair of an exogenously supplied CFTR target molecule carrying an F508 deletion in exon 10.

PTM

+

CFTR Target
(mini-gene)

Cotransfect PTM and Target molecules in HEK 293 cells
and detect repaired CFTR mRNA by RT-PCR.

Repaired
CFTR mRNA

Figure 1b

31304-A-B

Sheet 23 of 58

EXPERIMENT 3

Repair of endogenous CFTR
transcripts by exon 10 invasion
using a double splicing PTM

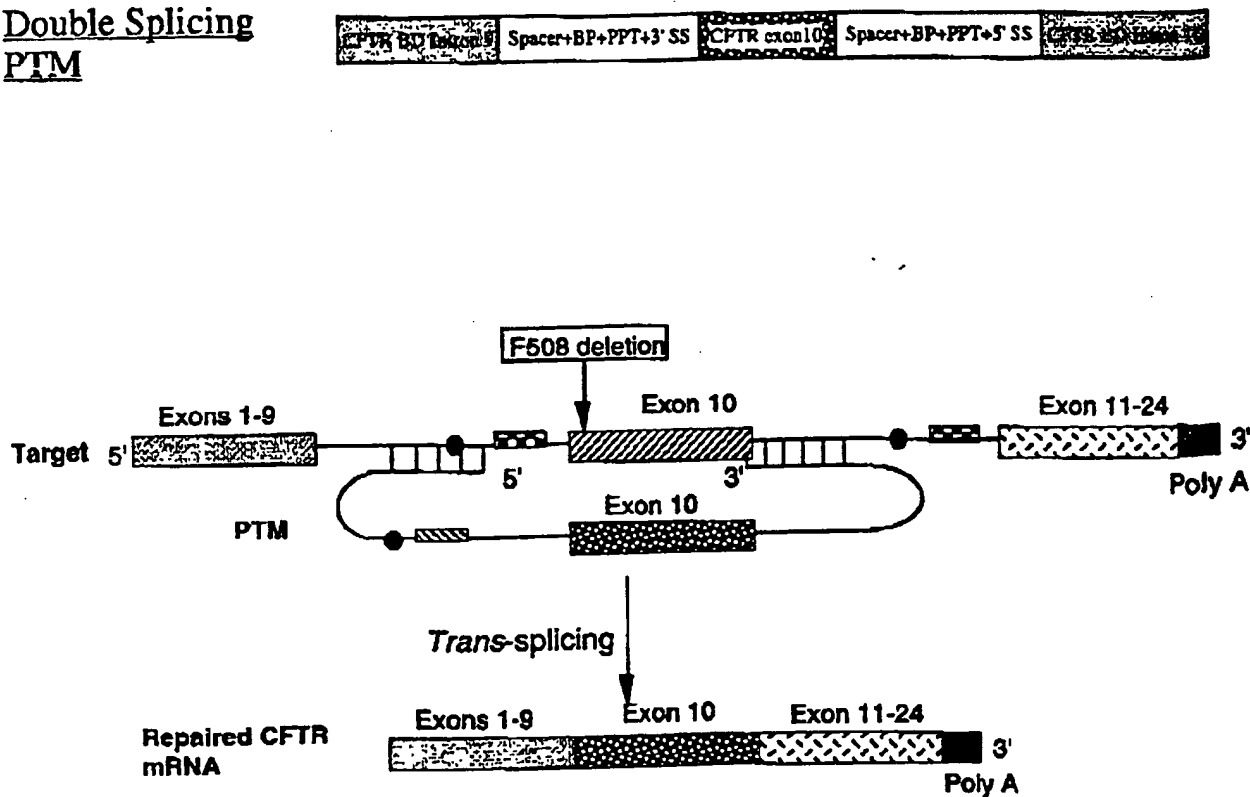
Double Splicing
PTM

Figure 17

31304 B-A

Sheet 24 of 58

Double Trans-splicing Specific Target

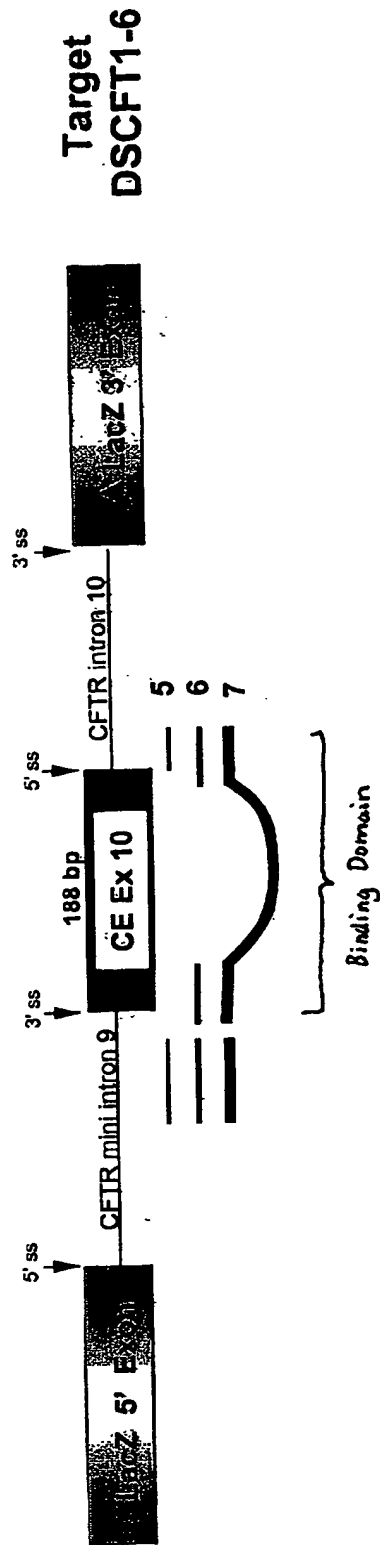
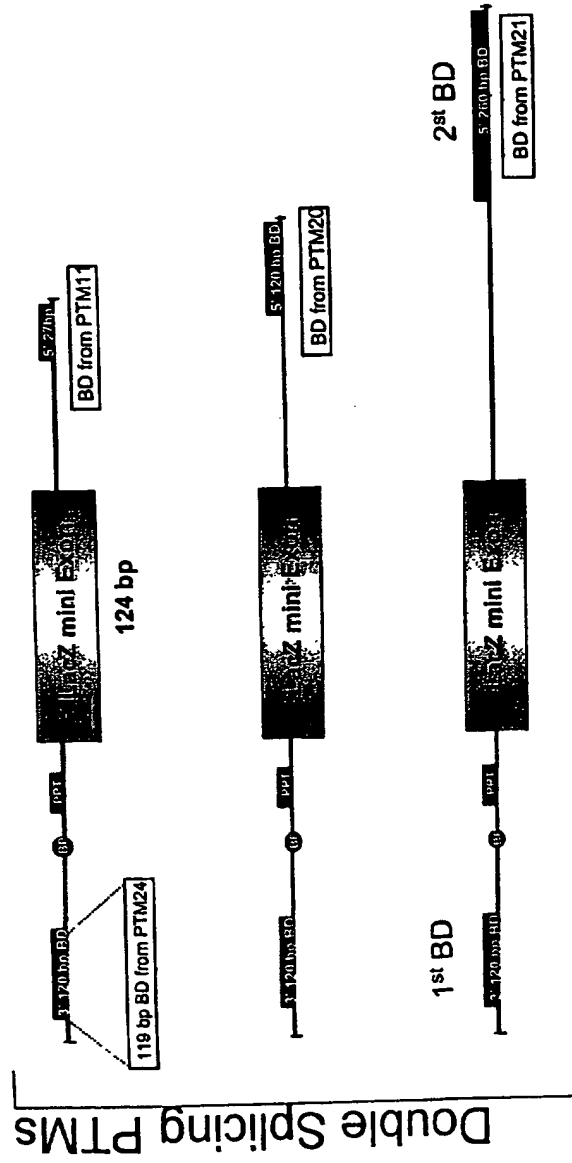


Figure 18

Sheet 26 of 58

Double Trans-splicing PTMs



DSPTM-5

PTM with 27 bp BD & masks 5' single splice site

DSPTM-6

PTM with 120 bp BD & masks both 5' & 3' splice sites

DSPTM-7

PTM with 260 bp BD masking both the ss & the entire CFTR Ex10

Figure 19

Double Trans-splicing β -Gal Model

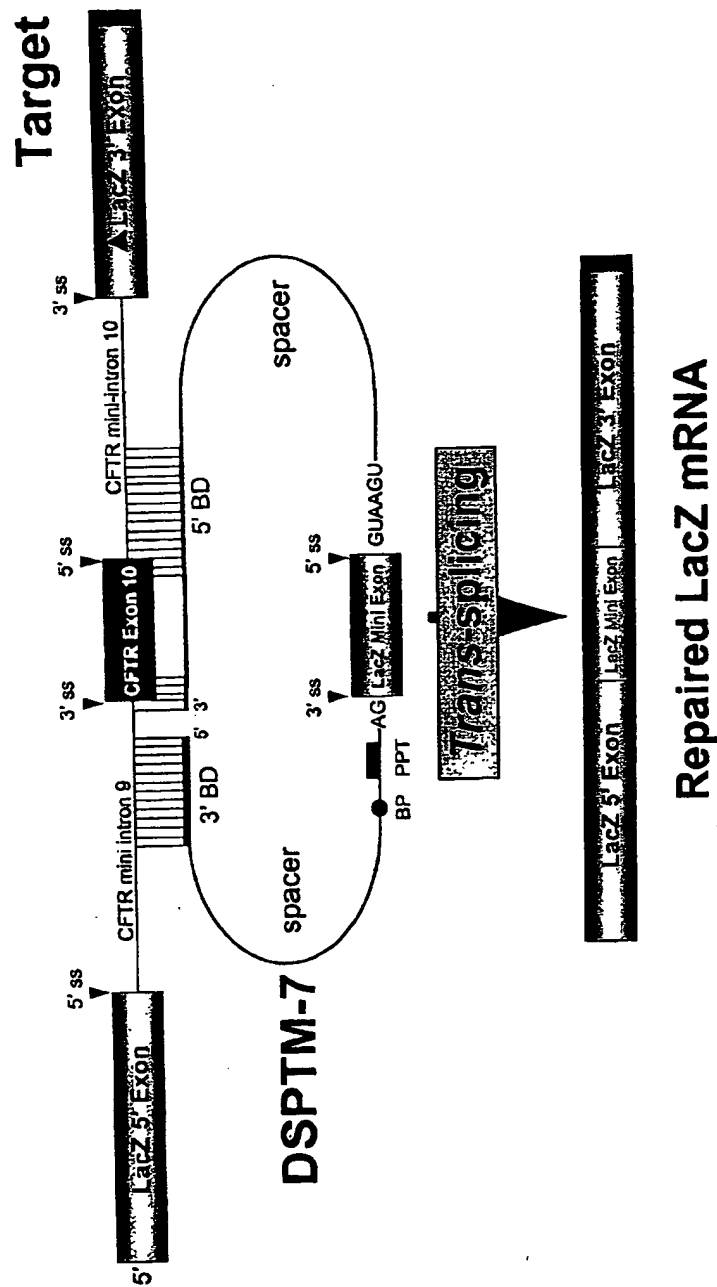
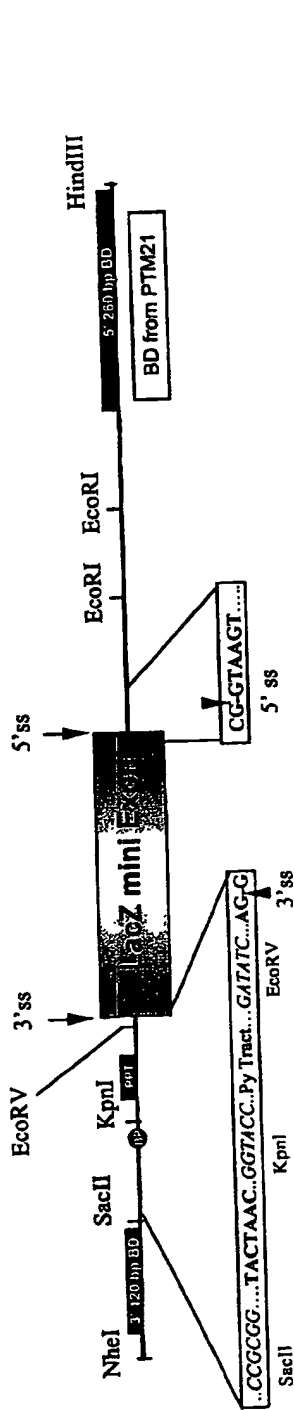


Figure 20

Important Structural Elements of DSPTM-7: (Double splicing PTM with all the necessary splice elements i.e. has both 3' and 5' functional splice sites and the binding domains)



(1) 3' BD (120 BP) : GATTCACCTTGCTCCAATTATCATCCTAAGCAGAAGTGATATCTTATTGTTAAAGATTCTATTAACATCTTTGATTC
AAAATATTTAAATACTCTCCTGTTTCATACTCTGCTATGCAC

(2) Spacer sequences (24 bp): AACATTATTATAACGTTGCTCGAA

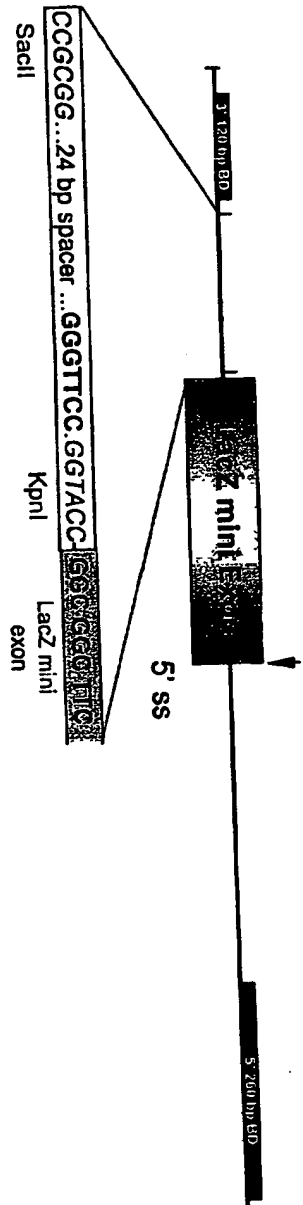
(3) Branch point, pyrimidine tract and acceptor splice site: TACTAAC T GGTAAC TCTTCTTTTTTTT GATATC CTGCAG GGG GGG
BP Kpn I PPT 3' ss LacZ mini exon

(4) 5' donor site and 2nd spacer sequence: TTAAGG GTAAAGT GTTATCACCGATATGTGTAACTGATTCCGGCCTTCGATACG
5' ss LacZ mini exon
CTAAGATCCACCGG

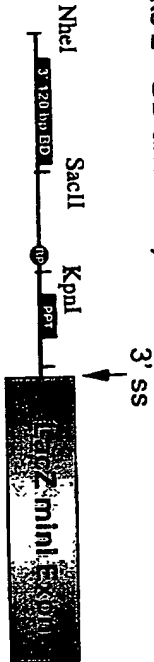
(5) 5' BD (260 BP) : TCAAAAAGTTTTCACATAATTTCTTACCTCTTGAAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATTCATTGGAA
ACACCAATGATTTTTCTTTAATGGTCCCTGGCATAATCCTGGAAACCTGATAACACAATGAATCTTCCACTGTGCTTAA
AAAAACCCTCTGAAATTCCTCCATTCTCCATAATCATCATTAACAACCTGAACCTCTGGAAATAAAACCCATCATTTAATCACTCA
TTATCAAAATCAGGC

Figure 21

DSPTM8 : (▲ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)



Mutants

Figure 22

Accuracy of Double Trans-splicing Reaction

Sheet 30 of 58

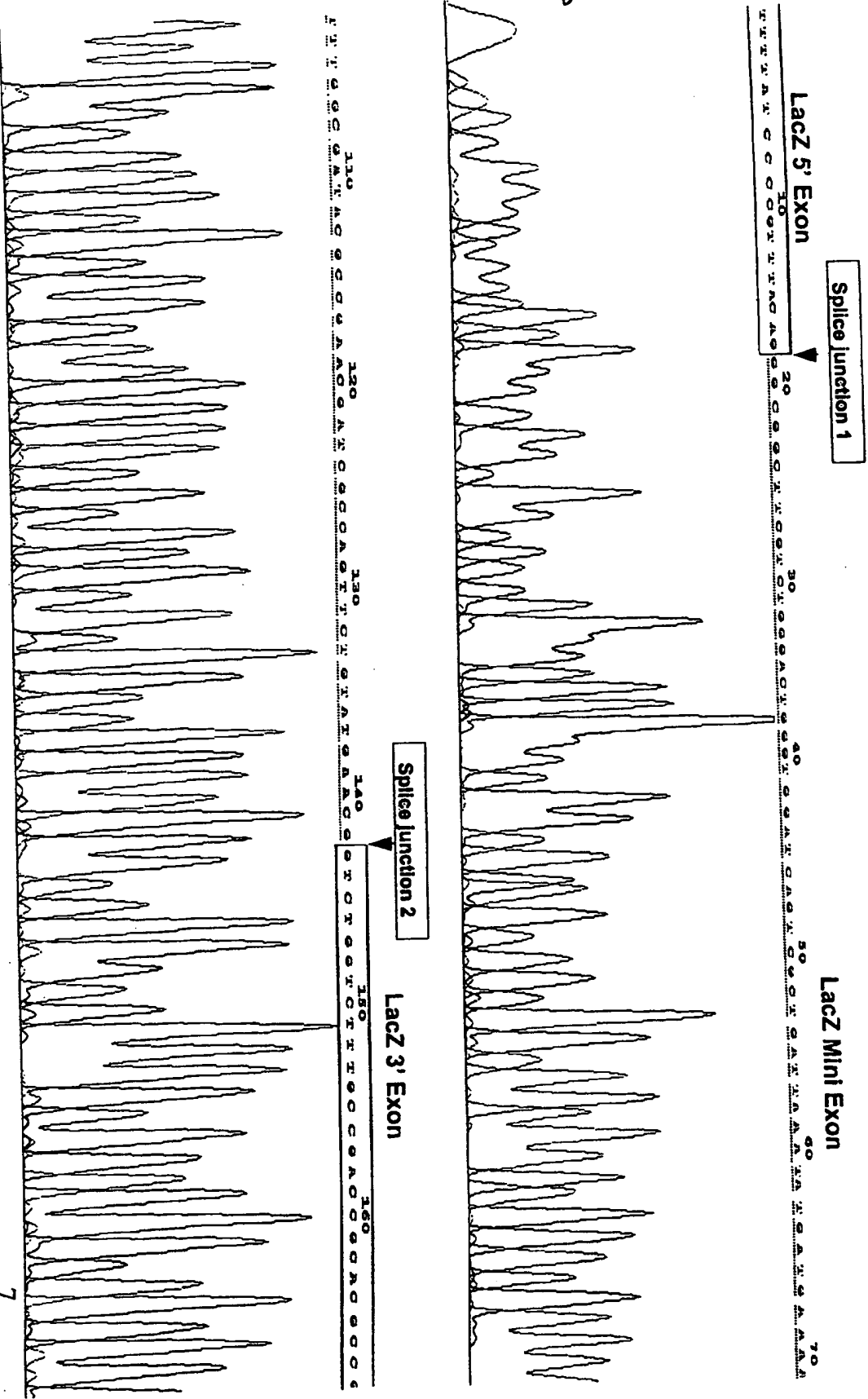


Figure 23

Double Trans-splicing Produces Full-length Protein

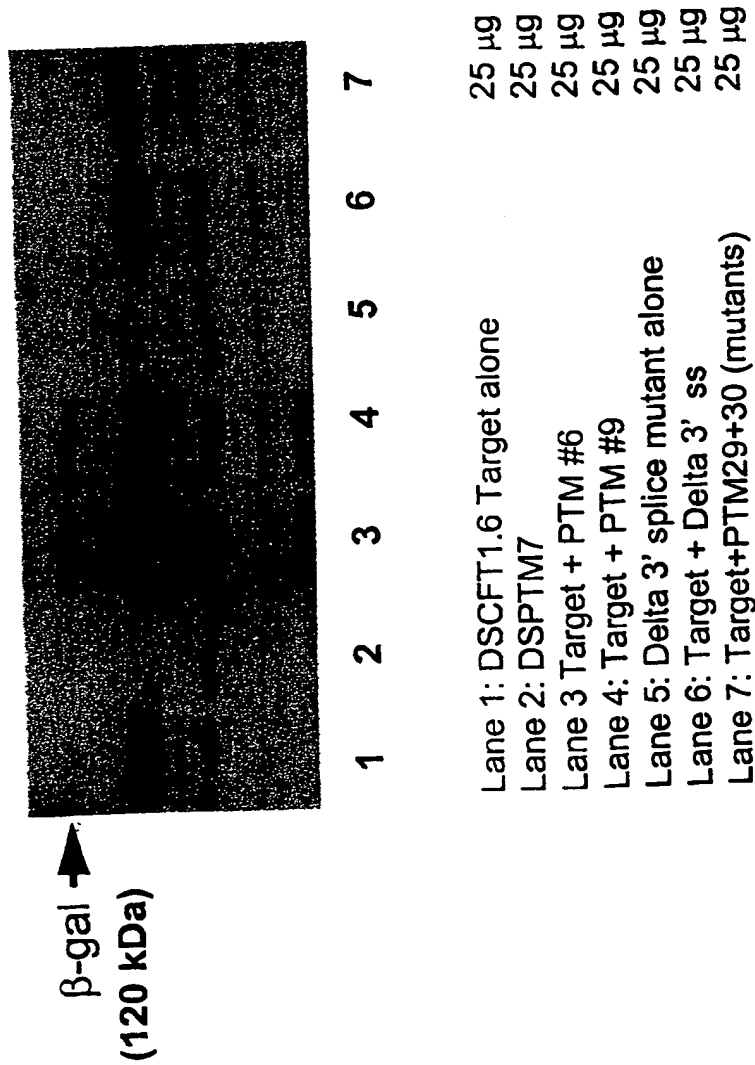
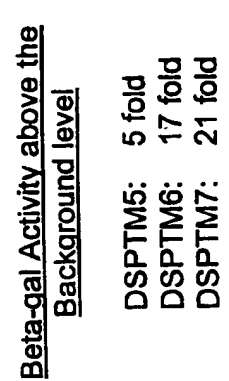


Figure 24

Atut 32 of 58



11

Restoration of β -gal activity is due to double RNA trans-splicing events

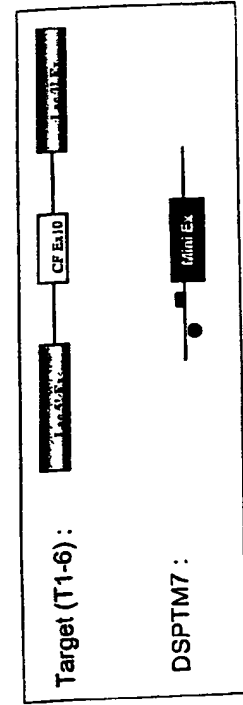
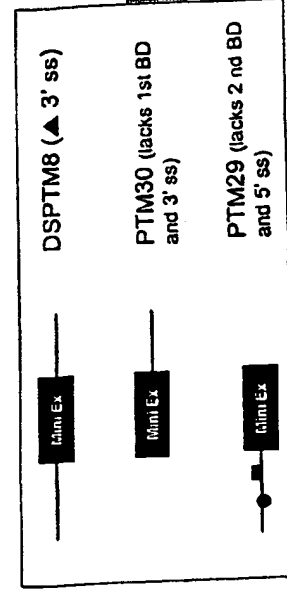
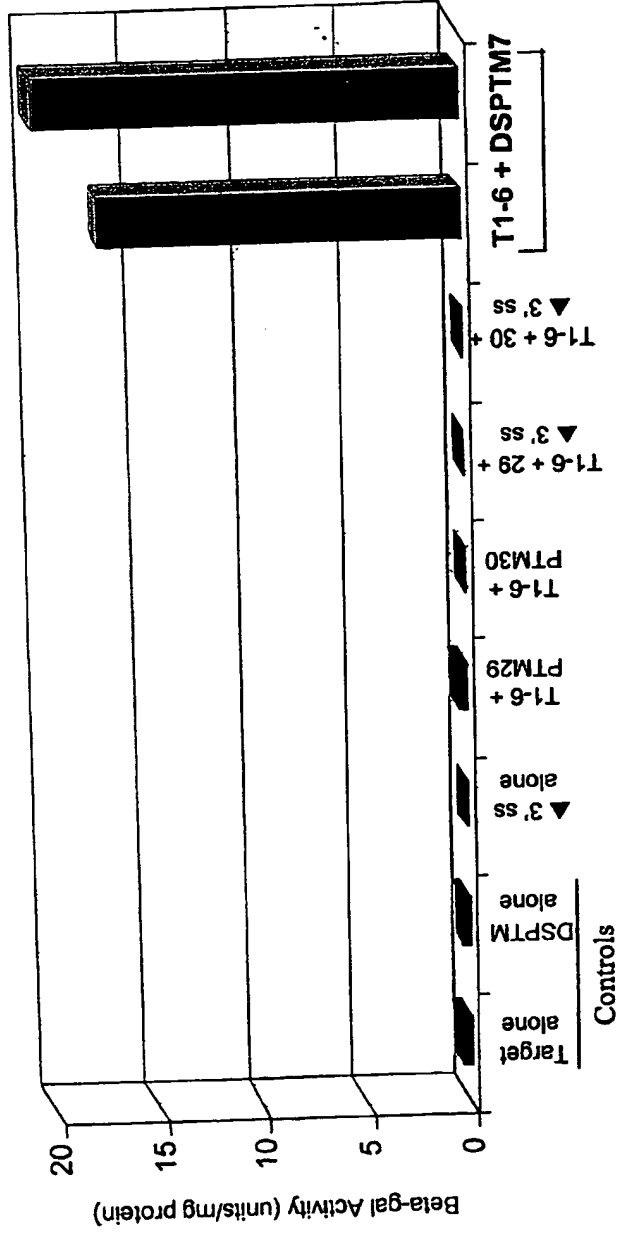
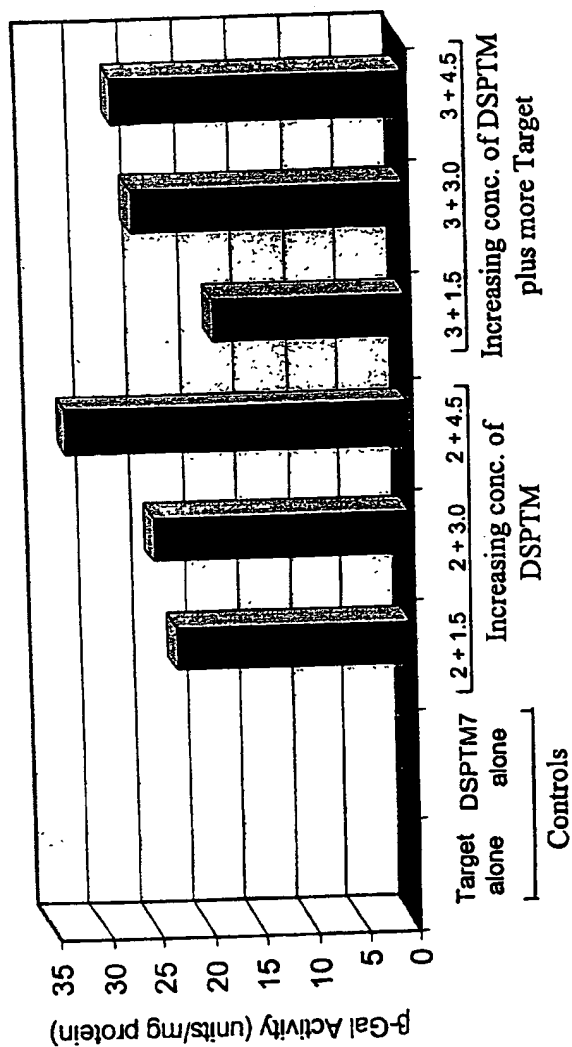


Figure 26

Sheet 33 of 58

Double Trans-splicing: Titration of Target & PTM



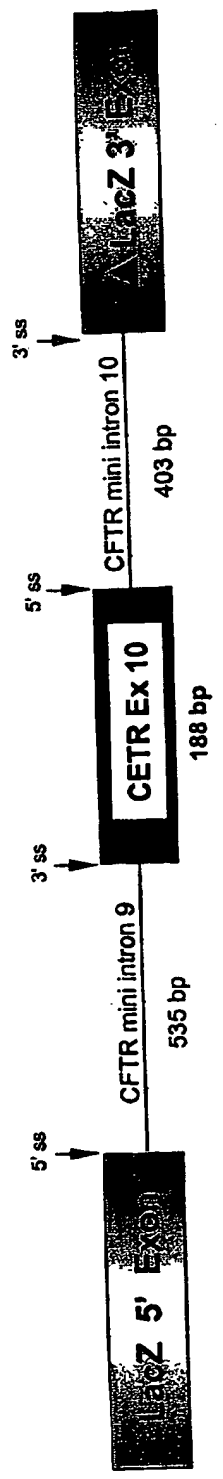
Sheet 34 of 58

The current level of beta-gal activity due to double trans-splicing is ~ 1-1.5% of the best single splice model (3' exon replacement)

Figure 27

11/25/2017 10:23:46 AM

DSCFT1-6 (Specific Target):



DSHCGT1 (Non-specific Target):

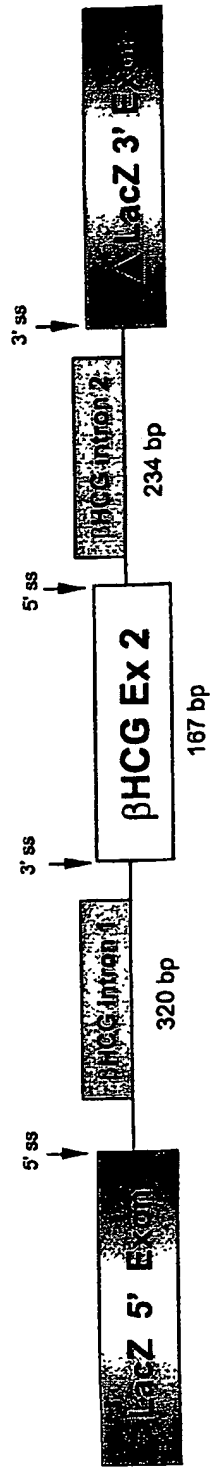


Figure 28

Sheet 35 of 58

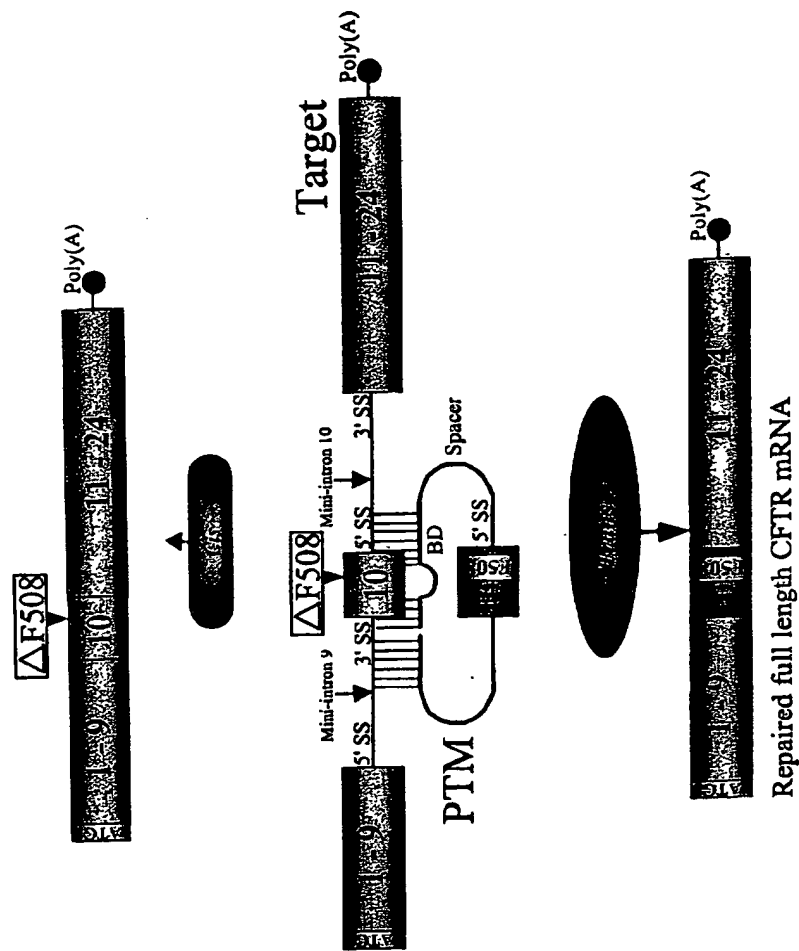
Sheet 36 of 58

Condition	Beta-gal Activity (units/mg protein)
DSHCG-T1 alone	1.1
DSHCG-T1 + DSPTM7	1.1
DSCF-T1.6 + DSPTM7	34

Figure 29

Sheet 37 of 58

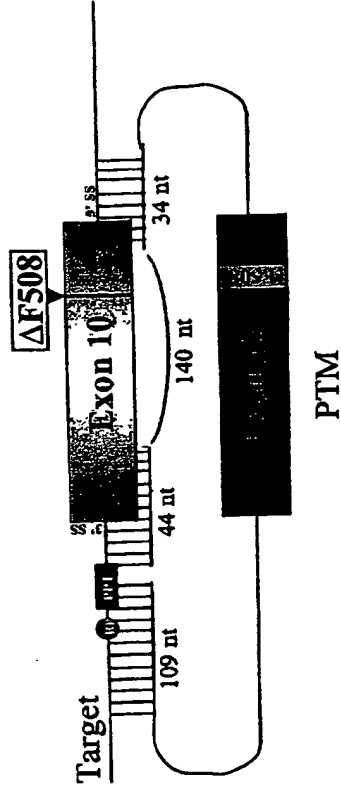
Replacement of a Single Nucleotide in a Schematic Diagram of a PTM Leading to a CFTR $\Delta F508$ Target



INTRONIN

Figure 30

PTM with a long binding domain masking two splice sites and part of exon 10 in a mini-gene target.



ACGAGCTTGGTCTCATGATGATCATGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAAACATTCCG
 GCCGCATCAGCTTTTGCAGCCAAATTCAGTTGGATCATGCCCGGTACCATCAAGGAGAAACATAAT
 CTTCCGGCTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCCTGTCAGTTGGAGGAG

MCU in exon 10 of PTM

88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain (bold and underlined).

Figure 31

INTRON

Sheet 38 of 58

Sheet 39 of 58

Sequence of a double
trans-spliced product

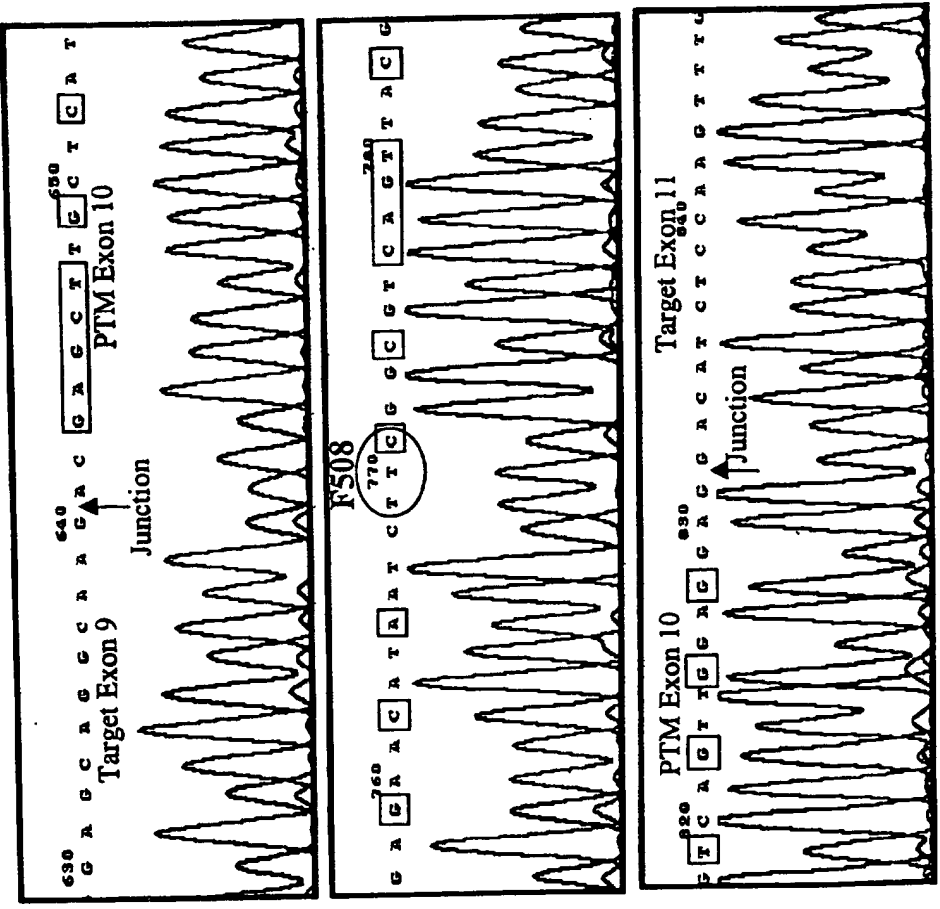


Figure 32

11

CFTR Repair: 5' Exon Replacement

Schematic diagram of a PTM binding to the splice site of intron 10 of an anti-gene target

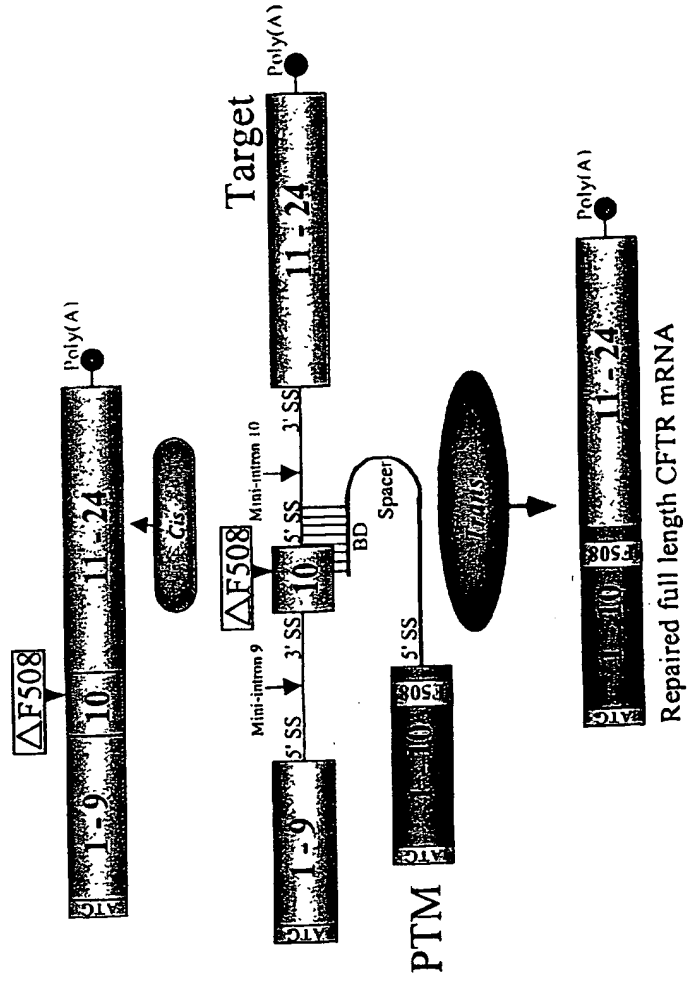
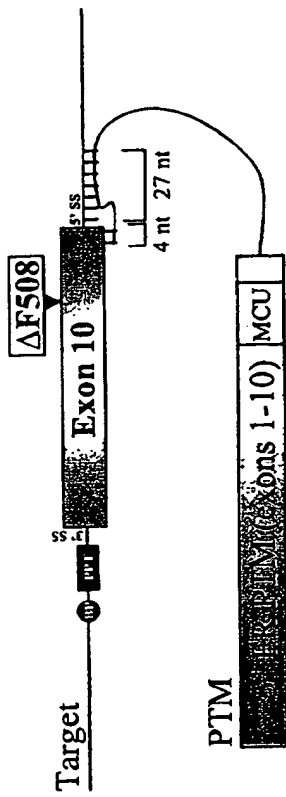
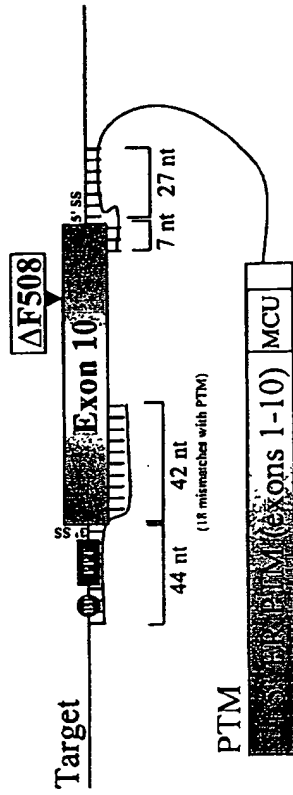


Figure 33

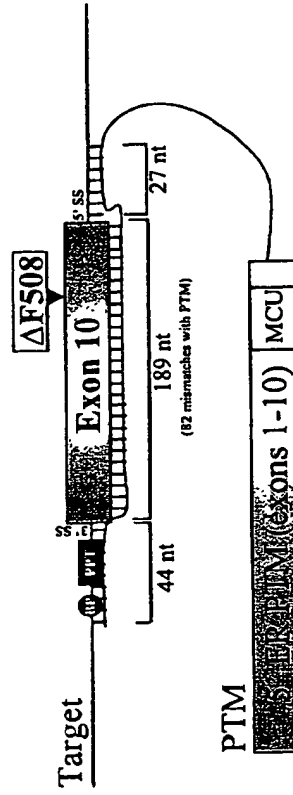
about 40 of 58



PTM with a short binding domain masking a single splice site in a mini-gene target.

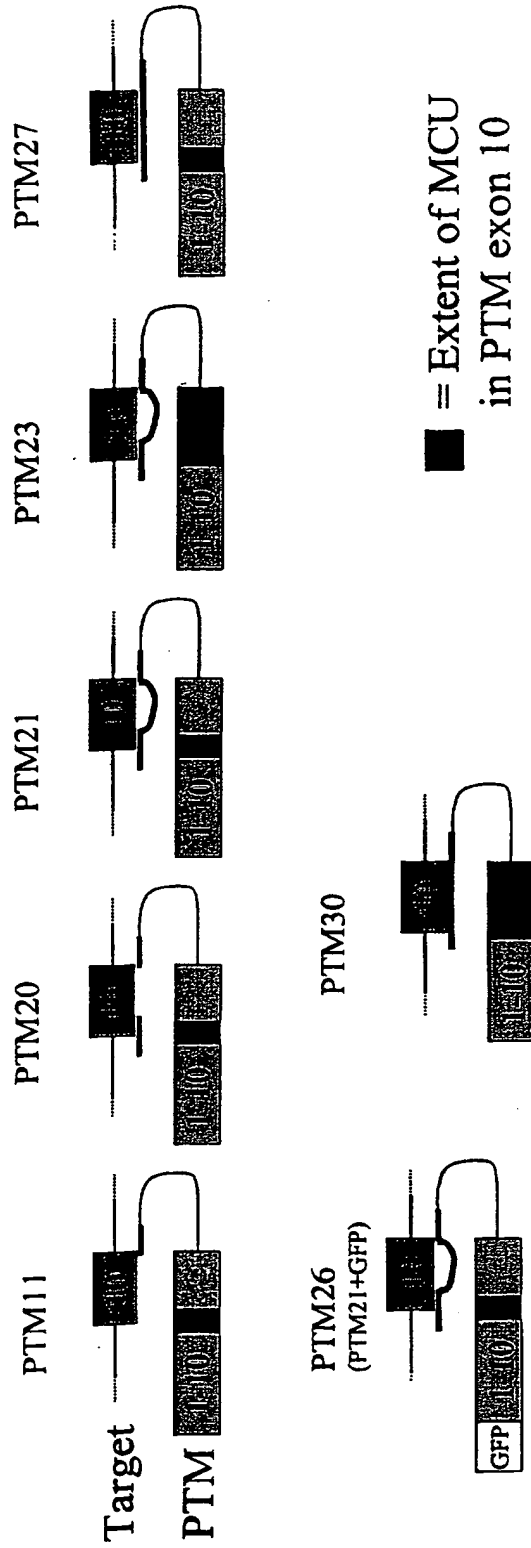


PTM with a long binding domain masking two splice sites in a mini-gene target.



PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

Figure 34



MCU in exon 10 of PTM

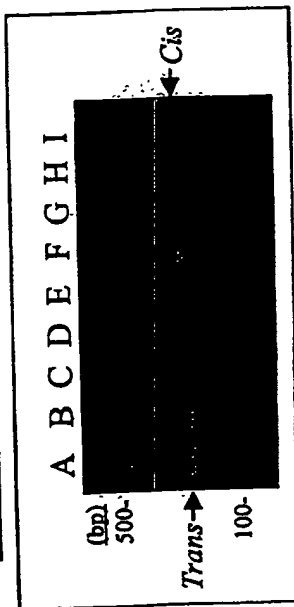
88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGCTCATGATGATGGGGGAGTTAGAACCAAGTGAAGGCAAGATCAAAACATTCCG
GCCGCATCAGCTTTTCAGCCCAATTTCAGTTGGATCATGCCCGGTACCATCAAGGAGAAACATAAT
CTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAAGGCCTGTCAGTTGGAGGAG

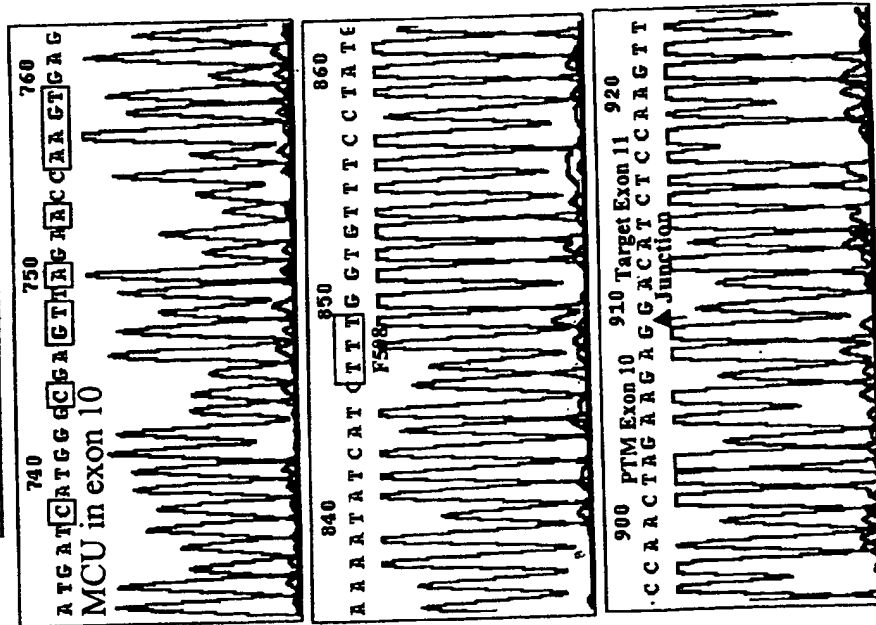
Figure 35

[illegible]

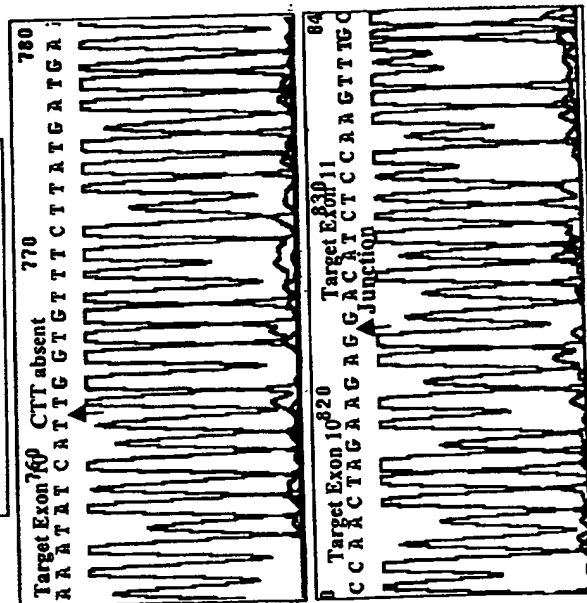
IRON



B. *Trans*-spliced product
[Primers CF93 + CF111]



A. Cis-spliced product
[Primers CF1 + CF111]



5

Figure 36

about 43 of 58

Sheet 44 of 58

A

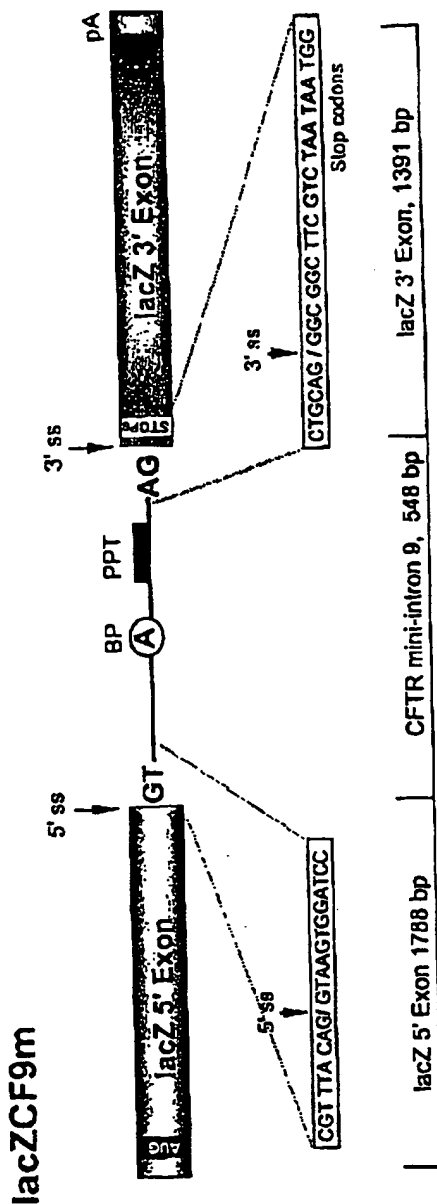


Figure 37 A

Sheet 45 of 58

B

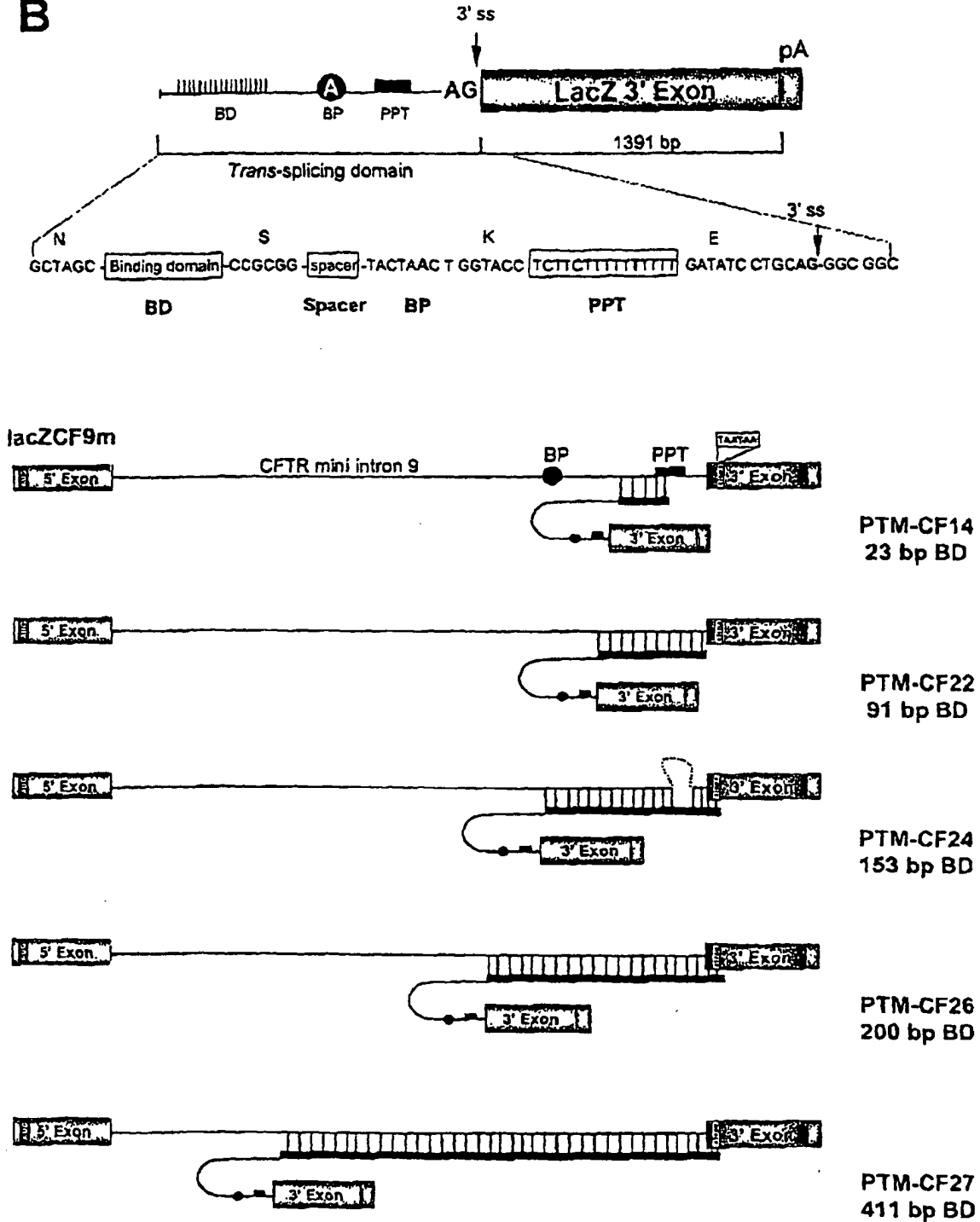


Figure 37B

C

Sheet 46 of 58

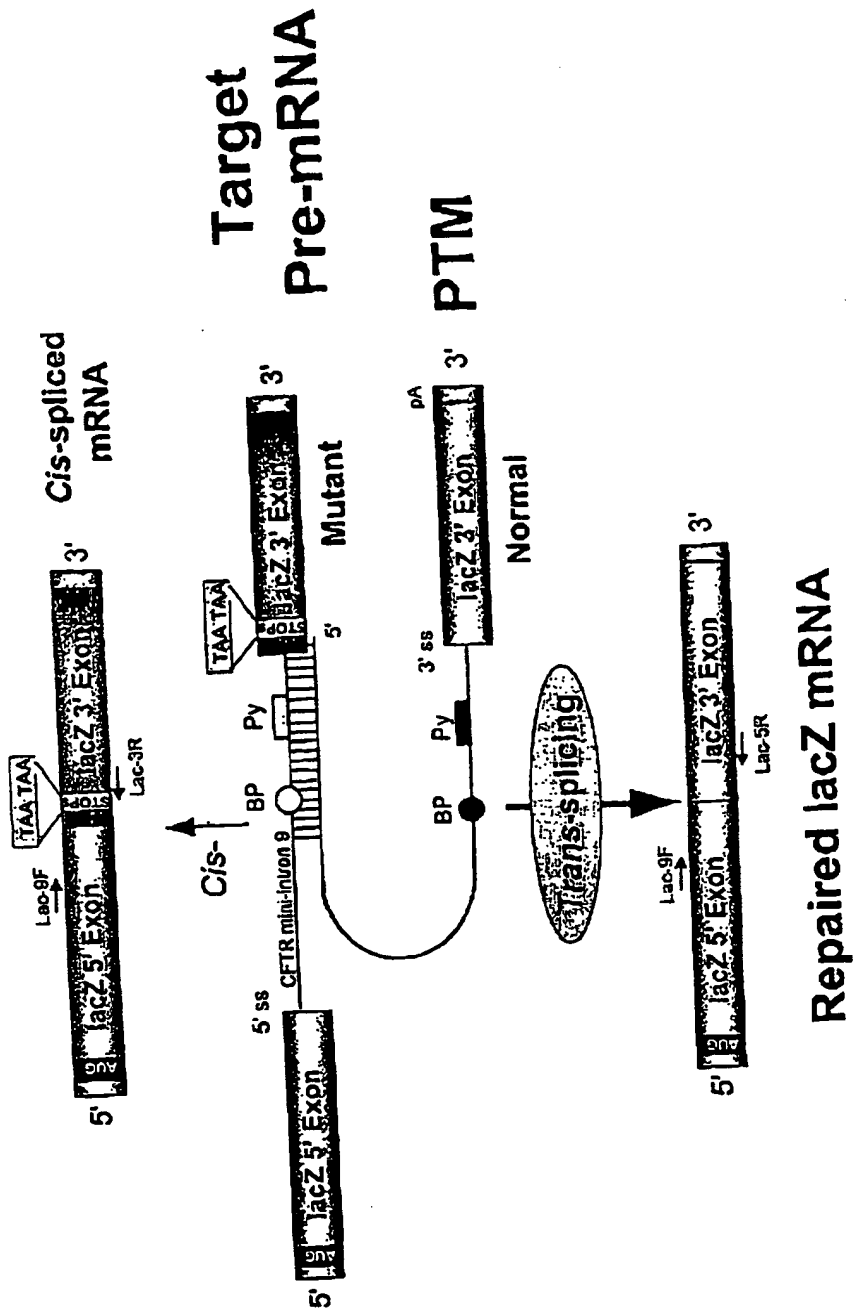


Figure 37C

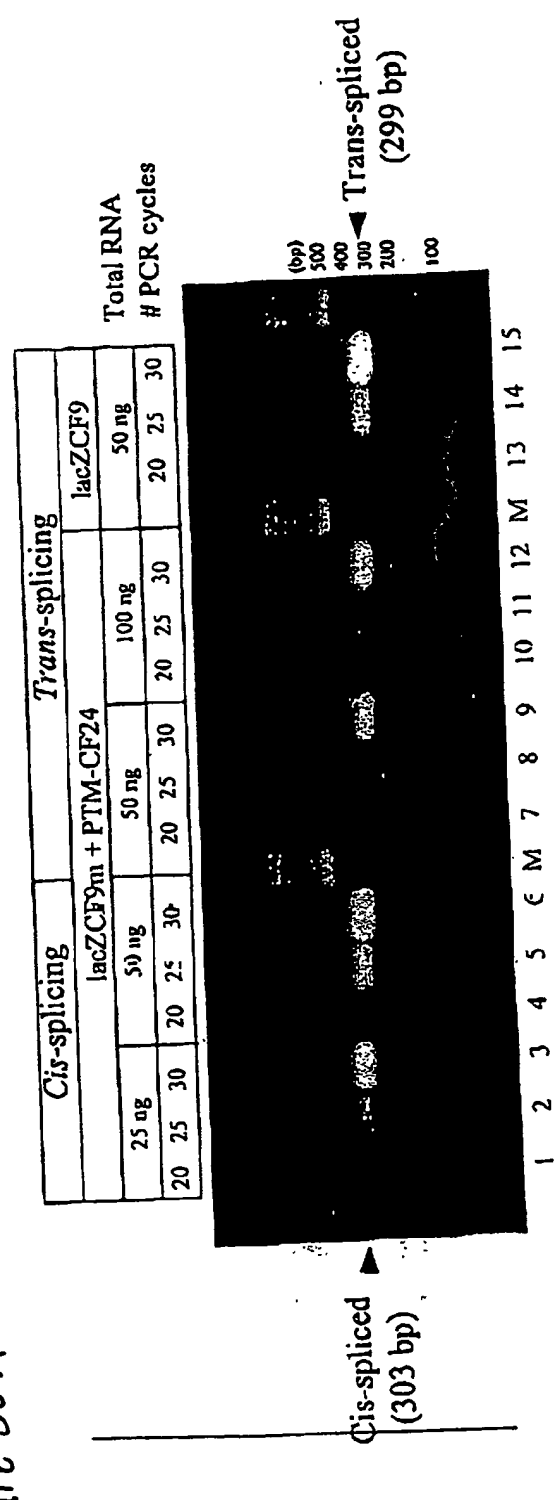
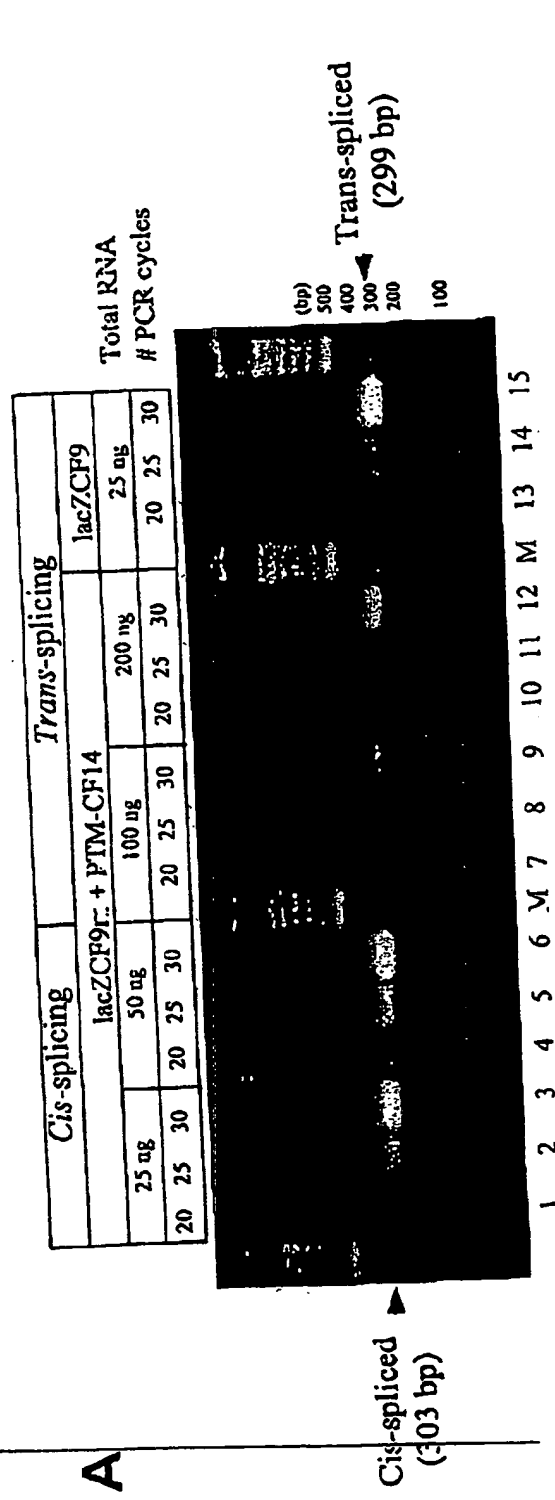


Figure 38 A

Sheet 47 of 58



But 48 of 58

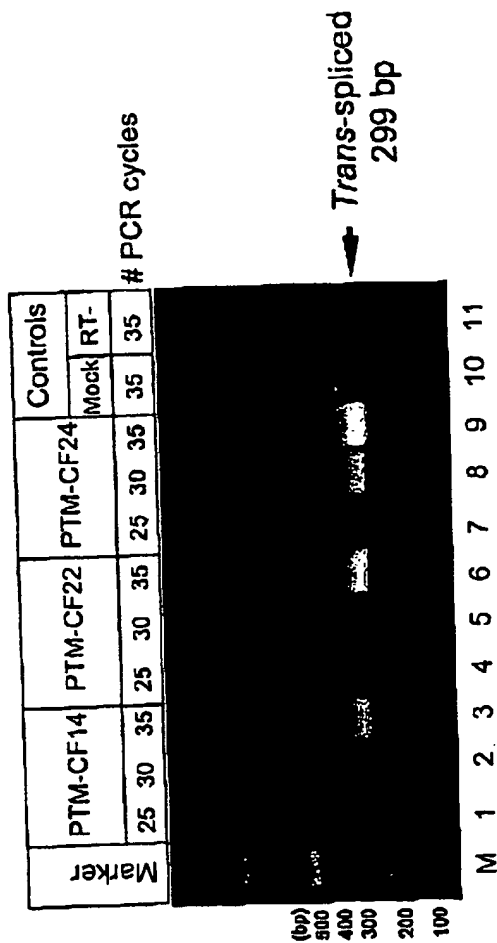


Figure 38B

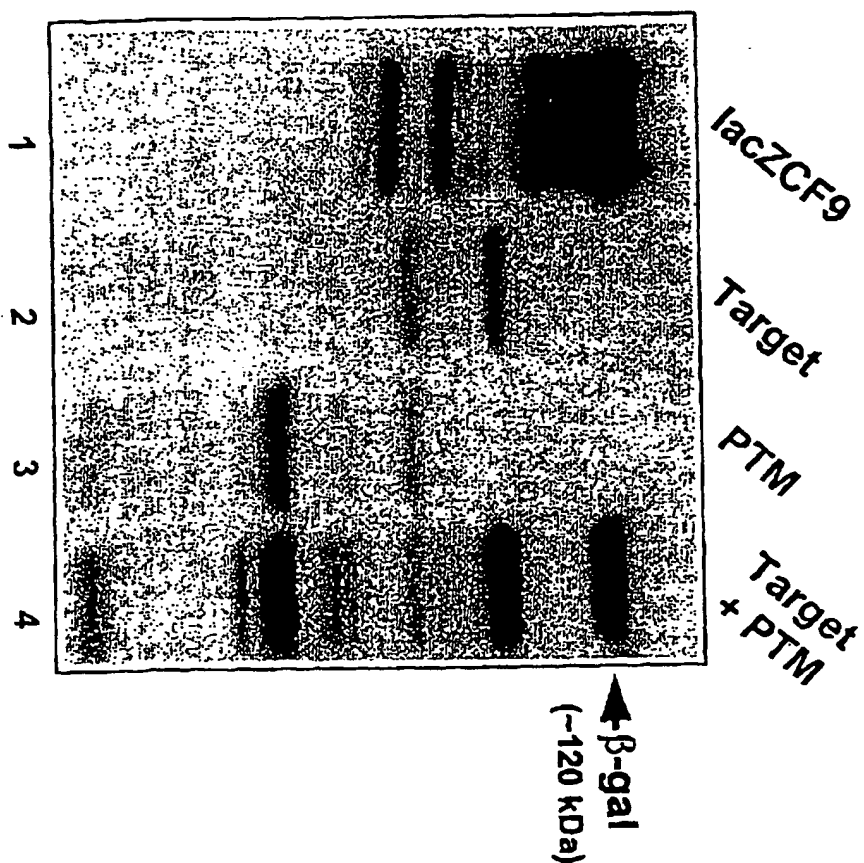
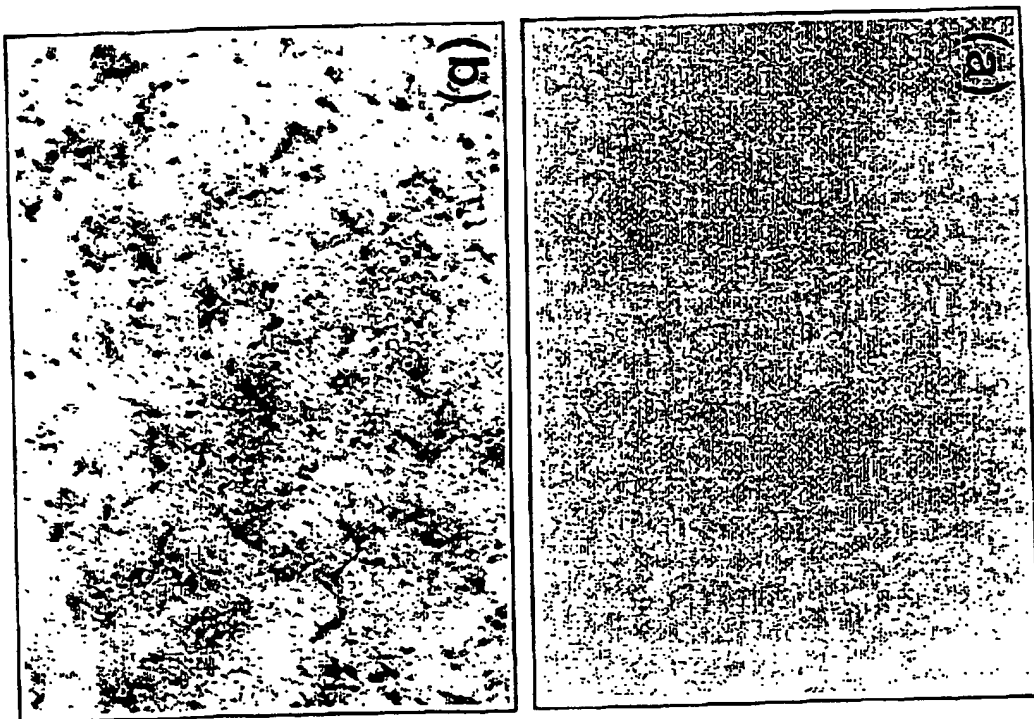


Figure 39

[illegible]

Figure 40A

A

[illegible]

Sheet 51 of 58

B

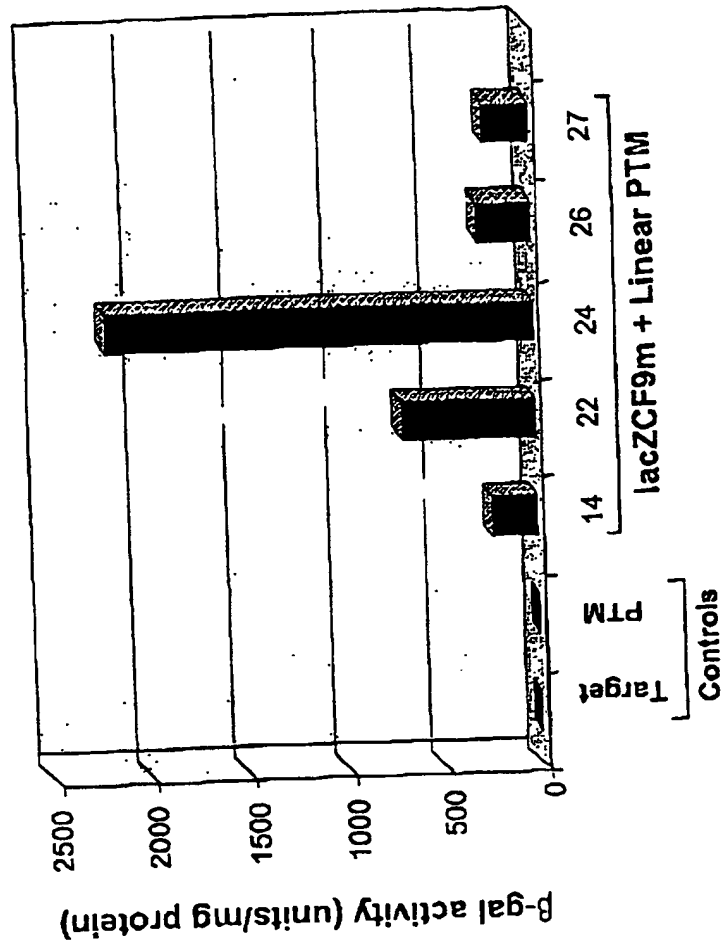


Figure 40B

[illegible]

Condition	β -gal activity (units/mg protein)
Mock	~0.5
Linear PTM	~45
PTM 14	~10
PTM 22	~12
PTM 24	~48

Figure 40C

Sheet 53 of 58

A

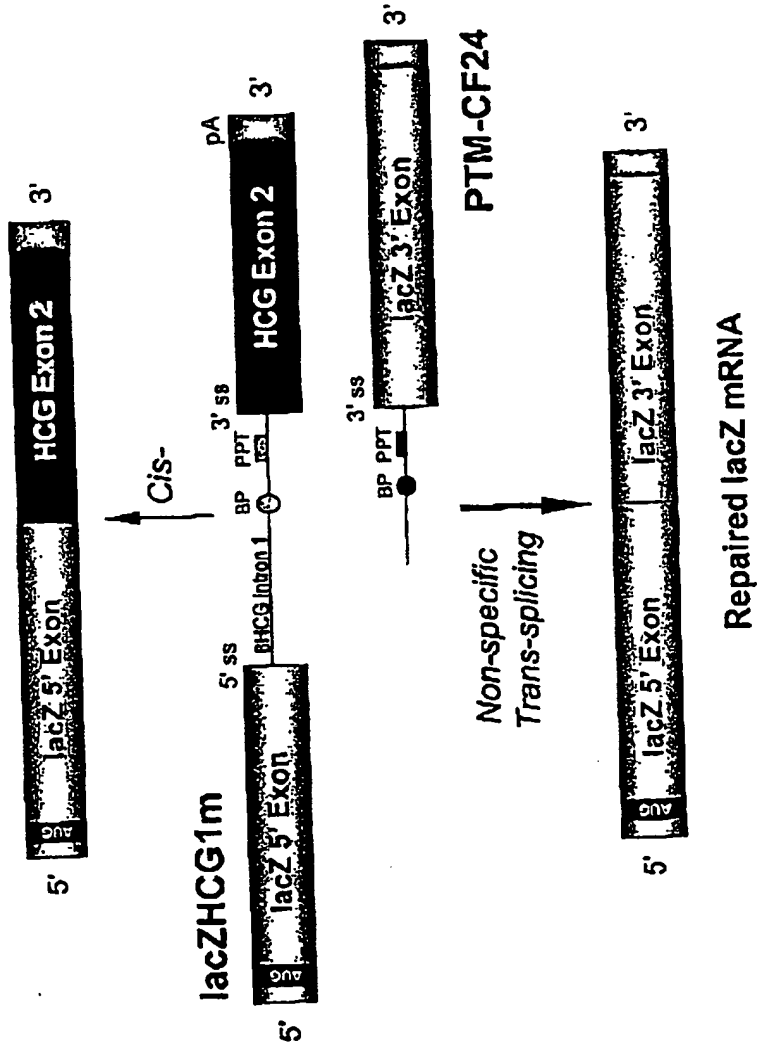


Figure 41A

Sheet 54 of 58

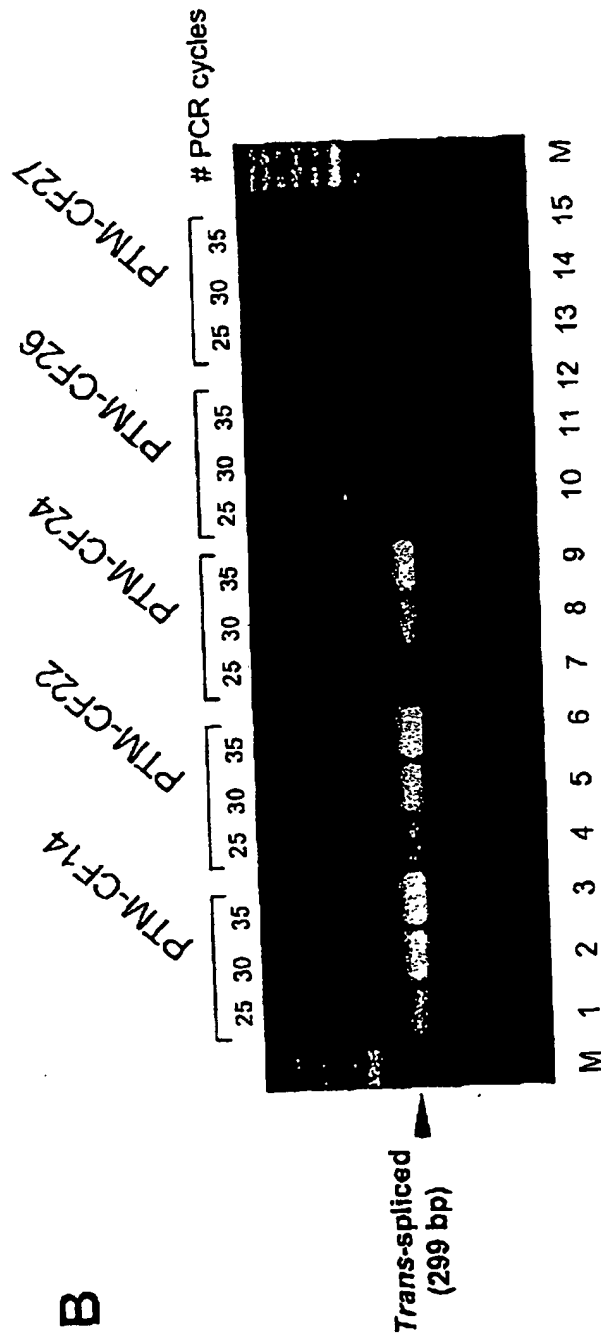


Figure 4KB

Sheet 55 of 58

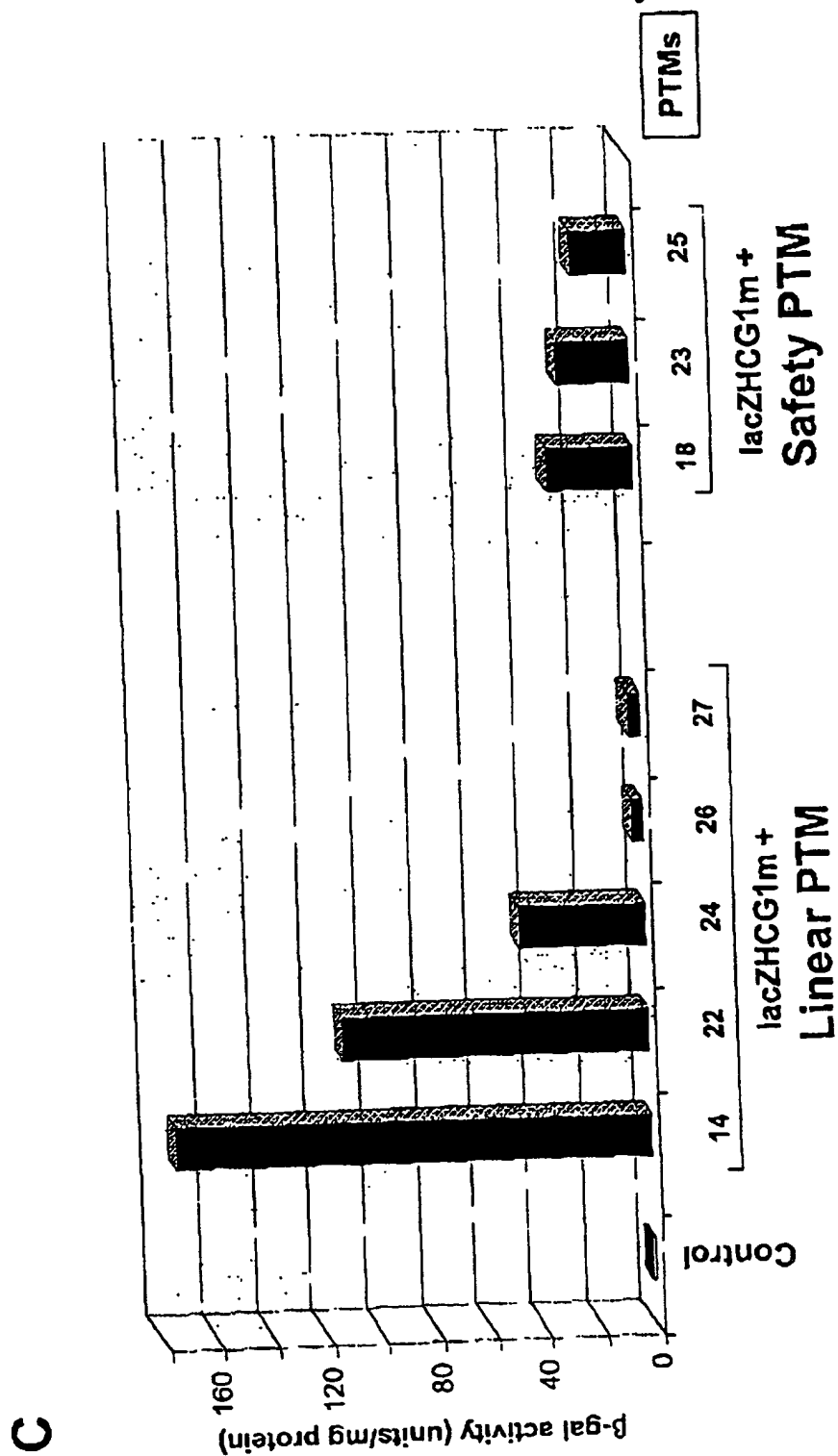


Figure 4C

Sheet 56 of 58

Exons 1-10

ATGCAGAGGTCGCCTCTGGAAAAGGCCAGCGTTGTCTCCAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG
GATACAGACAGCGCCTGGAATTGTCTAGACATATACCAAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT
GGAAAGAGAATGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTAATGCCCTTCGGCGATGTTTTTCTGG
AGATTTATGTTCTATGGAATCTTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAAGAATCA
TAGCTTCTCTATGACCCGGATAACAAGGAGGAACGCTCTATCGCGATTATCTAGGCATAGGCTTATGCCTTCTCTTTAT
TGTGAGGACACTGCTCCTACACCCAGCCATTTTGGCCTTCATCACATTGGAATGCAGATGAGAATAGCTATGTTTAGT
TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACCTTGTTAGTCTCCITT
CCAACAACCTGAACAAATTTGATGAAGGACTTGCAATTGGCACATTTCTGTGGATCGCTCCTTGGCAAGTGGCACTCCT
CATGGGGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGTGGACTTGGTTTCTGTATAGTCCCTGCCCCTTTTTCAG
GCTGGGCTAGGAGAAATGATGATGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGACTTGTGATTACCTCAG
AAATGATCGAGAACATCCAATCTGTTAAGGCATACCTGCTGGGAAGAAGCAATGGAAAAATGATTGAAAACCTTAAGACA
AACAGAACTGAAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTTCTCTCAGGGTTCTTT
GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCTCCGGAATAATTCACCACCATCTCATTCT
GCATTGTTCTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTGGAGCAATAAA
CAAAATACAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGAG
AATGTAACAGCCTTCTGGGAGGAGGGATTGCGGAATTATTTGAGAAAGCAAAACAAAACAATAACAATAGAAAAACTT
CTAATGGTGATGACAGCCTCTTCTTCACTAATTTCTCACTTCTTGGTACTCTGTCTGAAAGATATTAATTTCAAGAT
AGAAAGAGGACAGTTGTTGGCGGTTGCTGGATCCACTGGAGCAGGCAAGACGAGCTTGCTCATGATGATCATGGGCGAG
TTAGAACCAAGTGAAGGCAAGATCAAACATTCCGGCCGCATCAGCTTTTGACGCCAATTGAGTTGGATCATGCCCGGTA
CCATCAAGGAGAACATAATCTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAAGCCCTGTCAGTTGGA
GGAG

Trans-splicing domain

GTAAGATATCACCGATATGTGTCTAACCTGATTGGGCGCTTCGATACGCTAAGATCCACCGG
TCAAAAAGTTTTACATAATTTCTTACCTCTTCTTGAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCATTG
GAAACACCAATGATATTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCCTCCACTGT
GCTTAATTTTACCCTCTGAATTTCTCCATTTCTCCATAATCATCATTACAACGAACTCTGGAAATAAAACCCATCATT
ATTAACCTCATTATCAATCACGCT

Figure 42

[illegible]

Nhe I

GCTAGC-TGACGAAGCCGCCCTCAGCTCAGGATTCACCTTGCCTCCAATTATCATCTAGCAGAAGTGATA

Sac II

AC-CCGGG

Figure 43A

Sheet 58 of 58

Trans-splicing domain

AATAATGACGAAGCCGCCCTCACGCTCAGGATTCACTTGCCCTCCAATTATCATCCTAAGCAGAAGTGATATTTCTTA
TTTGTAAAGATTCTATTAACTCATTGATTCAAAATATTTAAATACTTCTGTTTCACCTACTCTGCTATGCACCCGC
GGAACATTATTATAACGTTGCTCGAATACTAAGTGTACCTCTCTTTTTTTTTTGATATCCTGCAG

Exons 10-24

ACTTCACCTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTGGAAGAATTTCACTTCT
GTTCTCAGTTTTCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTCCTATGATGAATATAGATA
CAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGGACATCTCCAAGTTTGCAGAGAAAGACAATATAGTTCTTGGAGAA
GGTGGAAATCACACTGAGTGGAGGTCAACGAGCAAGAAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTGTATT
TATTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAGAAAAAGAAATATTGAAAGCTGTGTCTGTAACTGATGGC
TAACAAAACCTAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAGAAAGCTGACAAAATATTAATTTGTCATGAAGGT
AGCAGCTATTTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCTCAAAAACCTCATGGGATGTGATT
CTTTTCGACCAATTTAGTGCAGAAAGAAGAAATCAATCTAAGTGAACCTTACACCGTTTCTCATTAGAAGGAGATGC
TCCTGTCTCCTGGACAGAAACAAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGGAAAAAAGGAAGAATTTCTATT
CTCAATCCAATCAACTCTATACGAAAATTTTCCATTGTGCAAAAGACTCCCTTACAAAATGAATGGCATCGAAGAGGATT
CTGATGAGCCTTTAGAGAGAAGGCTGTCTTAGTACCAGATTCTGAGCAGGGAGAGGCGATACTGCCTCGCATCAGCGT
GATCAGCACTGGCCCCACGCTTCAGGCACGAAGGAGGAGTCTGTCTGAACCTGATGACACACTCAGTTAACCAAGGT
CAGAACATTCACCGAAAGACAAAGCATCCACACGAAAAGTGTCACTGGCCCTCAGGCAAACTTGACTGAACCTGGATA
TATATTCAAGAAGGTTATCTCAAGAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCTT
TTTTGATGATATGGAGAGCATACCAGCAGTGACTACATGGAACACATACCTTCGATATATTACTGTCCACAAGAGCTTA
ATTTTGTGCTAATTTGGTGTCTTAGTAATTTTCTGGCAGAGGTGGCTCTTCTTGGTTGTGCTGTGGCTCTTGGAA
ACACTCTCTTCAAGACAAAGGAATAGTACTCATAGTAGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTC
GTATTATGTGTTTTACATTTACGTGGGAGTAGCCGACACTTTGCTTGTCTATGGGATTCTTCAGAGGTCTACCACTGGTG
CATACTCTAATCACAGTGTGCAAAAATTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTATGTCAACCTCA
ACAGTTTGAAGCAGGTGGGATTCTTAATAGATTCTCCAAAGATATAGCAATTTTGGATGACCTTCTGCCTCTTACCAT
ATTTGACTTCATCCAGTTGTTATTAATTTGTGATTGGAGCTATAGCAGTTGTGCGAGTTTACAACCTTACATCTTTGTT
GCAACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGAGCATATTTCTCCAAACCTCAGCAAACTCAAACAACCTGG
AATCTGAAGGCAGGAGTCCAATTTTCACTCATCTTGTACAAGCTTAAAAGGACTATGGACACTTCGTGCCTTCGGAGC
GCAGCCTTACTTTGAAACTCTGTTCCACAAGCTCTGAATTTACATACTGCCAACTGGTTCTTGTAACCTGTCAACACTG
CGCTGGTTCCAAATGAGAATAGAAATGATTTTGTGATCTTCTTCATTGCTGTTACCTTCATTTCCATTTTAAACAAG
GAGAAGGAGAAGGAAGATTGGTATTATCCTGACTTTAGCCATGAATATCATGAGTACATTGCAGTGGGCTGTAACTC
CAGCATAGATGTGGATAGCTTGATGCGATCTGTGAGCCGAGTCTTTAAGTTCATTGACATGCCAACAGAAGGTAAACCT
ACCAAGTCAACCAAAACCATAACAAGATGGCCAACCTCTCGAAAGTTATGATTATTGAGAATTACACGTGAAGAAAGATG
ACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGAATGCCATATTAGA
GAACATTTCTTCTCAATAAGTCTTGCCAGAGGGTGGCCCTCTTGGGAAGAACTGGATCAGGGAGAGTACTTTGTTA
TCAGCTTTTTTGGAGACTACTGAACACTGAAGGAGAAATCCAGATCGATGGTGTGTCTTGGGATTCAATAACTTTGCAAC
AGTGGAGGAAAGCCTTTGGAGTGATACACAGAAAGTATTTATTTTTCTGGAACATTTAGAAAAAAGCTGGATCCCTA
TGAACAGTGGAGTGATCAAGAAATATGGAAGTTGCGAGATGAGGTTGGGCTCAGATCTGTGATAGAACAGTTTCTGGG
AAGCTTGACTTTGTCTTGTGGATGGGGGCTGTGTCTAAGCCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTG
TTCTCAGTAAGGCGAAGATCTGTGCTTGTATGAACCCAGTGCTCATTGGATCCAGTAACATACCAAATAATTAGAAG
AACTCTAAAAACAAGCATTGCTGATTGCACAGTAATTTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAACAA
TTTTGGTCTATAGAAGAGAACAAAGTGGGAGTACGATTTCCATCCAGAACTGCTGAACGAGAGGAGCCTTTCCGGC
AAGCCATCAGCCCTCCGACAGGGTGAAGCTCTTCCACCAGGAACCAAGCAAGTGCAAGTCTAAGCCCCAGATTGC

Histidine tag Stop

TGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCTTCATCATCATCATCATCATTAG

Figure 43B